ORIGINAL ARTICLE

OLANZAPINE TRANSFER INTO SHEEP'S MILK. AN ANIMAL MODEL

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Abstract

A drug transfer into breast milk and inclusion in risk classes might be a cumbersome process, restricted by the small number of clinical cases available, especially for drugs with limited or infrequent use. To assess the excretion of olanzapine into breast milk we used a sheep animal model. Six sheep, with suckling lambs, were treated with 5 mg olanzapine daily, for 10 days, in order to reach steady state plasma concentration. Blood and milk samples were collected daily from the sheep, and an additional blood sample was taken from lambs on the 10th day. AUC and Css min values from plasma and milk were calculated to assess the milk/plasma ratio (M/P) and the relative infant dose (RD %) in order to compare these values with the available data from the literature. The determined minimum plasma concentrations were lower than those reported in humans for the same dose because of the pharmacokinetic differences between the two species, but the calculated M/P ratio values are comparable to those reported in literature which make this animal model suitable to predict the drug transfer into the breast milk.

Rezumat

Evaluarea transferului substanțelor medicamentoase în laptele matern și includerea acestora în clase de risc este dificilă datorită numărului limitat de rezultate provenite de la perechi mame/sugari disponibile, în special pentru medicamentele cu utilizare limitată. În vederea evaluării excreției olanzapinei în laptele matern am utilizat un model animal ovin. Șase oi, în lactație, cu miei, au fost tratate zilnic cu 5 mg olanzapină, timp de 10 zile, timp necesar atingerii concentrației plasmatice de echilibru. Probele de sânge și de lapte au fost colectate zilnic de la oi, la aceeași oră, iar în a ziua a 10-a o probă de sânge suplimentară a fost prelevată de la miei. Valorile minime AUC și Css din plasmă și lapte au fost calculate pentru a evalua raportul lapte/plasmă (L/P) și doza relativă pentru sugari (DR %), pentru a putea compara aceste valori cu datele disponibile din literatura de specialitate. Concentrațiile plasmatice minime determinate au fost mai mici decât cele raportate la om, pentru aceeași doză datorită diferențelor farmacocinetice dintre cele două specii, dar valorile raportului L/P sunt comparabile cu cele raportate în literatura de specialitate.

Keywords: olanzapine, drug safety, breastfeeding, milk, animal model

Introduction

Women suffering from psychiatric disorders, like schizophrenia, are required to undergo medical treatment throughout their lifetime, even when they are pregnant or breastfeeding. Olanzapine is the most commonly used antipsychotic drug during pregnancy and breastfeeding, although the safety data are controversial. In our previous research, we have shown that olanzapine, compared to other modern anti-psychotics, is safer to use during pregnancy that's why we aimed to also assess its safety during breast-feeding [13]. Because breastfeeding is the optimal form of infant nutrition, evaluating the amount of medication that an infant is exposed to is very important. When the medication is administered for a short time, the infant can be fed with a milk formula, but if the mother follows a long term treatment it is very difficult to estimate the daily child exposure. Breastfeeding is recommended either shortly before or immediately after taking the medication, when maternal blood concentration is the lowest.

The World Health Organization [16] classifies drug administration during breastfeeding as: compatible with breastfeeding (if there are no known or theoretical contraindications for their use), compatible with breastfeeding but the infant should be monitored for side-effects (if the drug could theoretically cause side-effects in the infant but have either not been observed to do so or have only occasionally caused mild side-effects), avoid if possible and the infant should be monitored for side-effects (if side-effects

in the infant have been reported), avoid if possible, because it might inhibit lactation, or avoid (drugs that can have dangerous side-effects on the baby).

Medicinal substances are classified in several categories with regard to their safety profile during lactation, as follows: L1 - safest, L2 - safe, L3 - moderately safe, L4 - possibly harmful, and L5- contraindicated [7].

Inclusion in any of the aforementioned risk categories is based on either the results from clinical studies performed on breastfeeding mothers or the data provided by paired drug concentrations determined in postpartum females and their breastfed children, respectively.

The drug transfer into breast milk depends on the properties of the drug (pKa, lipophilicity, protein binding and octanol: water partition coefficient). Plasma pH is 7.4 while milk pH is 7.0 ± 0.2, therefore, weak acid drugs have minimal diffusion into breast milk while the transfer is favourable for weak basic drugs (which can accumulate in milk) [4]. For mothers who need chronical treatment with basic drugs (drugs that are usually prescribed for CNS - central nervous system), child exposure problems are significant.

To estimate the amount of drug transferred to the suckling infant, the M/P ratio (based on the areas under the respective concentration-time curves) can be calculated using the mathematical formula proposed by Begg E. J. *et al.* [2].

For basic drugs:

$$\ln(M/P) = -0.09 + 2.54\ln(M_u/P_u) + 0.79\ln(f_{u,p}) + 0.46\ln K,$$

$$M_u/P_u = [1 + 10^{(pKa-7.2)}]/[1 + 10^{(pKa-7.4)}],$$

$$K = (0.955/f_{u,m}) + (0.045 \times \text{milk:lipid } P_{app}),$$

where: M_u/P_u = milk:plasma unbound drug concentration ratio, $f_{u.p}$ = fraction of drug unbound in plasma, $f_{u.m}$ = fraction of drug unbound in milk, P_{app} = apparent partition coefficient at pH 7.2 [11]:

$$f_{u.m} = f_{u.o}{}^{0.45}/[(6.94 \times 10^{-4})^{0.45} + f_{u.p}{}^{0.45}],$$

where: $f_{u,p}$ = 1 - PB, PB - plasma protein binding value.

For substances in which the apparent partition coefficient is unknown, it can be approximated from $\log D_{7.2}$ (the octanol-water distribution coefficient at pH 7.2) [11]:

$$\log P_{app} = -0.88 + 1.29 \times \log D_{7.2}.$$

This formula has its own limitation as between predicted M/P ratio (based on physiochemical characteristics of drugs) and calculated M/P ratio (based on real data available in literature) there is no significant correlation as suggested by Larsen L. A. *et al.* [12].

Ethical reasons forbid controlled studies to appreciate the transfer of olanzapine through the blood-milk barrier in humans; therefore, we aimed to asses drug's excretion into breast-milk using an animal model.

Materials and Methods

Animals. Six sheep weighing between 38 and 52 kg (46.3 ± 7.3 kg) in the early lactation period, each having a suckling lamb, were enrolled in the experiment. Lambs were kept with their mothers, all animals being clinically healthy and parasite free. Hay and water were provided *ad libitum*. The sheep were housed with full protection against wind, rain, extremes of temperature and humidity.

The experiments were performed in accordance with international ethical guidelines (Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes) and the protocol was approved by the Ethics Committee of the University of Medicine and Pharmacy of Târgu Mureș, Romania.

Experimental model. Each ewe received 5 mg olanzapine daily (oro-dispersible tablet) *per os,* for 10 days (because there are no available data regarding the safety of olanzapine in this animal species, they received an olanzapine dose as small as possible, but also comparable to human doses). Individual blood samples (from the jugular vein in K_3EDTA vacuum tubes) and milk samples (collected by manual milking) were taken daily at the same time, before the next dose (corresponding to the lowest plasma concentration that a drug reaches before the next dose -$C_{ss\ min}$). At steady-state, additional blood samples from lambs were taken.

Blood plasma was separated by centrifugation at 3500 rpm at 4°C, then plasma and milk samples were kept frozen at -20°C until analysis.

Analytical method. An HPLC/MS/MS method was used to determine the drug concentration in biological samples. The method was validated for sheep plasma and milk (unpublished data). The method was not validated for lamb plasma due to the required quantity of biological material which is not acceptable for ethical reasons. After protein precipitation with formic acid 0.1% in acetonitrile, the biological samples were analysed using a LC-MS/MS system 1100 series (Agilent Technologies, USA). The chromatographic separation was performed on Zorbax SB-C18 100 x 3 mm, 3.5 µm analytical column (Agilent Tech.) and the mobile phase consisted of a mixture of acetonitrile and formic acid 0.1% (40:60) and it was delivered at a flow rate of 0.4 mL/min.

Quantification was performed by mass spectrometry in MRM mode on a QQQ detector with ESI operated in positive mode.

Results and Discussion

Assessment of the infant safety of drugs administered to the mother during breastfeeding periods must take into account the fact that the amount of substance the baby is exposed to might vary, depending on the drug concentration in milk, the amount of milk and the moment it is ingested, therefore DID (daily infant dose) can be calculated using certain mathematical formulas proposed by Sachs H. C. [15]:

$$DID\ (mg/day) = C_{milk}\ (mg/mL) \times V_{milk}\ (mL),$$

where: DID - daily infant dose, C_{milk} - average drug concentration in milk, V_{milk} - volume of milk ingested in 24 hours. V_{milk} is typically estimated to be 150 mL/kg/day for a breastfed infant.
For lambs, we initially determined the milk volume by weighing the lambs before and after suckling and we calculated an average of 0.15 L/kg/day milk ingestion, similar to that reported in humans.
The relative dose to which the infant is exposed can be also calculated:

$$RD\ (\%) = [DID\ (mg/kg/day)/DMD\ (mg/kg/day)] \times 100,$$

where: RD - relative infant dose, DID - daily infant dose, DMD - daily maternal dose.
Olanzapine was identified in the plasma of ewes on the second day of treatment and in milk on the third day.
In Table I we present AUC_{x-y} values (calculated using the mixed log-linear method, x representing the first biological sampling and y-last treatment day), minimum steady-state concentration ($C_{ss\ min}$) from milk and plasma and the M/P radio (milk/plasma ratio – calculated for each sampling time at steady state). The individual results were processed as averages ± SD.

Table I

Average $C_{ss\ min}$ and AUC_{ss} in plasma and milk – olanzapine 5 mg daily oral dose (x = 1, y = 10)

$C_{ss\ min}$* plasma (ng/mL)	AUC_{ss}* plasma ((ng/mL)/day)	$C_{ss\ min}$* milk (ng/mL)	AUC_{ss}* milk ((ng/mL)/day)	M/P ratio	RD %
0.45 ± 0.21	4.03 ± 1.89	0.22 ± 0.02	1.71 ± 0.43	0.47 ± 0.40	0.43 ± 0.11

*$C_{ss\ min}$ is defined as minimum steady state drug concentration during a dosing interval, **AUC_{ss} is defined as area under the curve during a dosing interval at steady state.

Drug plasma concentration fluctuations between the maximum and minimum concentrations following each dose after repeated administration had the same variability. Pharmacokinetic parameters were assessed using Kinetica 5.1 SP1 (Thermo Fisher Scientific). A non-compartmental model was used to calculate values used for determining M/P ratio and to calculate infant drug exposure *via* breastfeeding.
The individual concentration-time data ("spaghetti plot") after repeated dose administration from plasma are presented in Figure 1.
Figure 2 shows the olanzapine mean concentration levels from plasma samples.

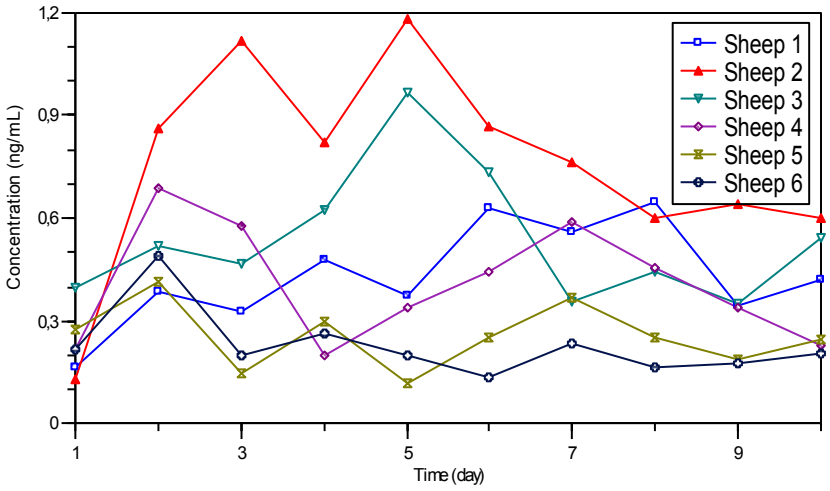

Figure 1.
Sheep plasma concentrations ("spaghetti plot") after 5 mg olanzapine (n = 6)

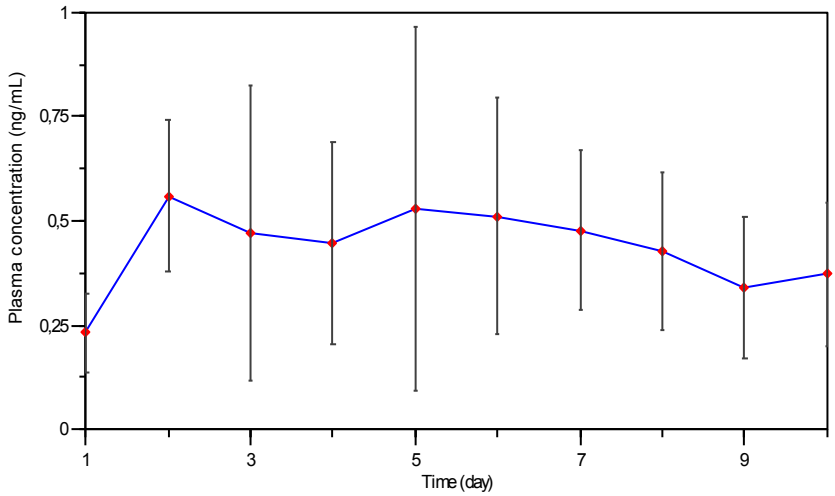

Figure 2.
Olanzapine mean plasma level (n = 6)

The individual olanzapine concentration-time data ("spaghetti plot") after repeated dose administration and the mean concentration levels from milk samples are presented in Figure 3 and Figure 4 respectively.

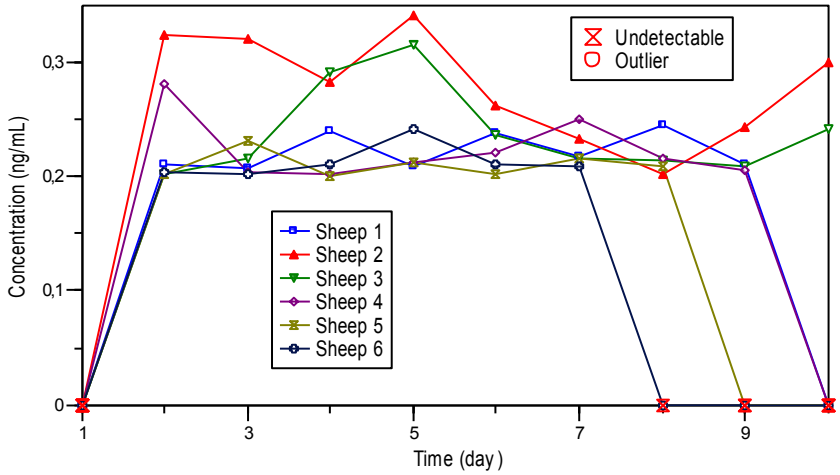

Figure 3.
Sheep milk concentrations ("spaghetti plot") after 5 mg olanzapine (n = 6)

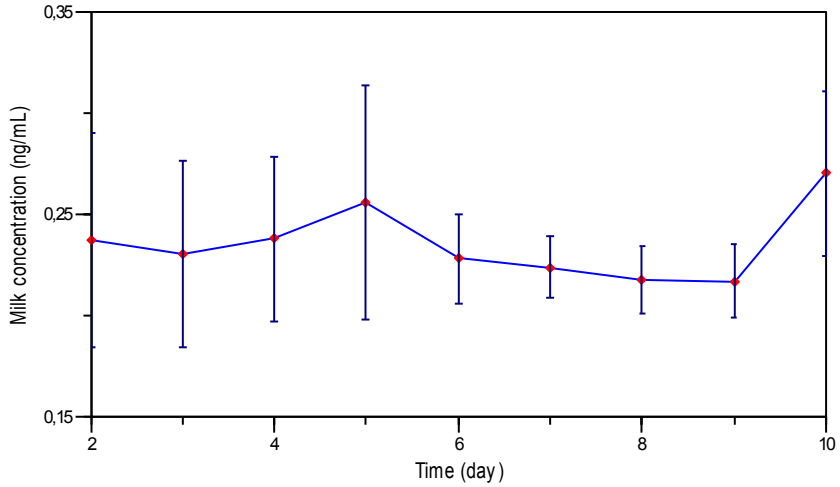

Figure 4.
Olanzapine mean milk level (n = 6)

At steady-state, additional blood samples were collected from suckling lambs, but olanzapine plasma values were below the lower limit of quantification (LLOQ = 0.1 ng/mL) of the analytical method.

No side-effects (sedation, lethargy, suckling difficulties) were noted in the suckling lambs by the veterinarian, the animals being considered clinically healthy.

Olanzapine was chosen in this study as it is a basic substance but highly controversial in scientific literature regarding its transfer into breast milk. Due to its pharmacologic profile, even at the highest doses olanzapine was not associated with persistent elevations of prolactin level; it has no influence on milk secretion [1, 7].

Olanzapine is classified in risk class L3. As there are insufficient data in literature to draw a conclusion regarding safety during breastfeeding, prescription is made based on the benefit to the mother and the severity of the illness.

The M/P olanzapine values calculated using Atkinson and Begg's equations are 0.38 (pKa = 7.24, logP = 2.02, protein binding = 93%) as suggested by Croke S. *et al.* [2, 6]. Our study suggested an M/P ratio ranging between 0.1 - 0.9 (mean 0.46) for olanzapine, a value similar to that reported using Begg's E. J. formula, but also suggested by measurements of M/P ratio from breastfeeding mother/child pairs (mean 0.46, range 0.2 - 0.8), calculated at 11 - 23 hours post-dose [6].

A sheep animal model was chosen for various reasons: their body weight and size approximates to that of humans, they adapt well to laboratory situations, blood pH is comparable between sheep and humans (≈ 7.4), which is extremely important to establish the ratio between the ionized and unionized form of drugs [8]. The blood pH in sheep being similar to humans, drug transfer through blood-milk barrier is comparable, but mature milk pH differs between the two species being approximately 7.2 in women and 6.6 in sheep, which can influence the quantity of olanzapine that can return into the blood [14]. The exposure of the breastfed infant/lamb to the maternal medication depends on several factors.

Olanzapine plasma levels obtained in ewes are significantly lower compared to those reported in humans; these differences can be explained by the pharmacokinetic differences of chemicals in the two species. There are differences in drug absorption between the two species due to some particularities of the gastro-intestinal tract. In ruminants, the stomach is compartmented in *rumen*, *reticulum*, *omasum* and *abomasul* (the real glandular compartment), while humans the stomach is monogastric [3]. Because drug absorption depends on the ionized/unionized form ratio, the local pH and the pKa of the drug are very important. In humans, drug absorption occurs in the stomach or intestines and it can be approximated using the Henderson-Hasselbach equation and the pKa:

$$\log [\text{unionized form}]/[\text{ionized form}] = \text{pKa} - \text{pH}.$$

Throughout the ruminant digestive tract, the pH in the *rumen* varies between 5.5 and 6.5, which is favourable for the absorption of weakly basic drugs. The pKa of olanzapine is 7.34, as such, it should be absorbed from the *rumen* and there should be no differences regarding its absorption between sheep and humans, but there could be differences in the amount of substance absorbed in 24 h due to some metabolic differences between the two species. Sheep possess a complex digestive system in which food takes 25 - 35 hours to pass through the gut and is exposed to microbial fermentation in the *rumen*. Sieving processes are involved, with large particles being regurgitated for re-mastication by the process of rumination. The length and volume of the gastro-intestinal tract in ruminants is much more pronounced then in humans resulting in longer passage and usually delayed absorption after oral formulations. A large volume of the ruminal fluid dilutes the drugs and decreases their rate of absorption delaying their effect [10]. The pH of milk is more acid (6.8 - 7.2) than that of plasma (7.4), consequently, the percent of the unionized form is different and milk/plasma (M/P) drug concentration ratios can be predicted utilizing the pKa value proposed by Begg E. J. *et al.* [2].

Although pH values of sheep's gastrointestinal tract are suitable to absorb substances with basic character (the *rumen* pH is 6 - 7 and it is comparable to that found in the humans' small intestine), it is unknown to what extent drug stability in the *rumen* is affected, since there are microorganisms involved in digestion.

Milk olanzapine concentrations were 0.22 ± 0.02 ng/mL while plasma concentrations were more variable (0.45 ± 0.21 ng/mL), which can be explained by the limited capacity of the active substance to cross the plasma-milk barrier due to its great affinity for plasma proteins (93%).

Olanzapine is metabolized by CYP1A2. Since there are limited data regarding liver microsomal drug-metabolizing enzyme system in the sheep it is difficult to assess how the first phase's metabolism reactions are arising at this species.

Conclusions

Olanzapine is characterized by lipophilicity, marked basic character, being non-ionized at pH plasma values which allows an easy passage through the blood-brain barrier, but also reflected through an extensive milk excretion.

Even if slightly alkaline lipophilic nature facilitates penetration of the blood-milk barrier, the levels in mammary glandular secretion are the result of two opposite trends: the lipophilic character favours the transfer into the milk, since it is an emulsion of lipids in a solution containing proteins (lactalbumin,

lactoferrin, casein) and sugars (lactose); on the other hand, marked lipophilicity leads to a large volume of distribution (Vd) which is reflected by low plasma levels.

Plasma olanzapine concentrations in sheep are smaller compared with those found in humans (case reports, there are no controlled trials during breastfeeding in literature), which can be explained by interspecies pharmacokinetic differences.

The blood-milk barrier penetration showed a limited transfer for olanzapine, the M/P ratio being below par (according to international classification substances having an M/P ratio < 1 are considered compatible with breastfeeding). Lack of adverse effects and very low plasma concentrations detected in lambs reflect a benefit/risk ratio favourable to this medication during breastfeeding.

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