DOI 10.1007/s10517-023-05723-3
Bulletin of Experimental Biology and Medicine, Vol. 174, No. 4, February, 2023

BIOPHYSICS AND BIOCHEMISTRY

Functioning of P-Glycoprotein during Pregnancy in Rabbits

N. M. Popova, A. V. Shchulkin, I. V. Chernykh, P. Yu. Mylnikov, and E. N. Yakusheva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 174, No. 10, pp. 434-438, October, 2022
Original article submitted July 1, 2022

The level P-glycoprotein (Pgp) in organs of pregnant rabbits and its content and activity in the placental barrier at different stages of pregnancy were studied. An increase in Pgp content in the jejunum on days 7, 14, 21, and 28 of pregnancy in comparison with this parameter non-pregnant females was revealed by ELISA; in the liver, Pgp content was higher on day 7 and tended to increase on day 14; in the kidney and cerebral cortex, Pgp content was higher on day 28 of pregnancy in parallel with an increase in serum progesterone concentration. We also observed a decrease in Pgp content in the placenta on days 21 and 28 of pregnancy in comparison with day 14 and a decrease in Pgp activity in the placental barrier, which was confirmed by enhanced penetration of fexofenadine (Pgp substrate) through the barrier.

Key Words: *P-glycoprotein (Pgp, ABCB1-protein); protein content; protein activity; fexofenadine*

P-glycoprotein (Pgp, ABCB1 protein) transports a wide range of lipophilic substrates from the cell to the extracellular space or organ cavities [1]. Pgp limits the absorption of substrates in the intestine, prevents their penetration into the trans-barrier organs through the blood–tissue barriers (blood–brain, blood–testis, and blood–placenta), and promotes excretion of endo- and xenobiotics into bile and urine in the liver and kidneys, respectively [1,2].

Pgp functioning in the placental barrier protects the fetus from potentially toxic substances and drugs, substrates of Pgp [3]. The penetration of substrates to the fetus is determined by not only permeability of the placenta, but also pharmacokinetic processes in the mother's body (absorption, distribution, and excretion of substances affecting the synthesis and activity of Pgp in organs and tissues).

Ryazan State Medical University, Ministry of Health of the Russian Federation, Ryazan, Russia. ***Address for correspondence:*** p34-66@yandex.ru. N. M. Popova

At the moment the scientific literature presents works devoted to the study of Pgp functional activity and content and the expression of genes encoding the transporter-protein in the placental barrier in various animal species, however, Pgp functioning of other localizations during pregnancy has not been studied.

Rabbits are recommended as the test system for studying Pgp functioning *in vivo* [4]. Our aim was to study the content of Pgp in the organs of pregnant females and Pgp content and functioning in the placental barrier at various stages of pregnancy.

MATERIALS AND METHODS

The study was performed on 45 female Soviet Chinchilla rabbits weighing 3000-3500 g. The study protocol was approved at a meeting of the Commission for the Control over the Keeping and Use of Laboratory Animals (No. 11, January 28, 2018).

The study was performed in two series. In series I, Pgp content in organs of female rabbits was studied

on days 7, 14, 21, and 28 of pregnancy (n=5 for each time point). The control group consisted of intact non-pregnant female rabbits; the organs were taken immediately after sacrifice. In series II, Pgp functioning in the placental barrier was studied on days 14, 21, and 28 of pregnancy. The control group in this series consisted of intact non-pregnant female rabbits.

The first day of pregnancy was considered the first day after mating. Pregnancy was diagnosed by the increase in progesterone concentration in the blood serum, as well as by visual signs and palpation data. In series I, the serum concentrations of progesterone, estradiol, and testosterone were determined at the specified time by radioimmunoassay using standard test system (Immunotech). To measure the levels of the hormone, the blood was taken from females before pregnancy and on the specified days of the experiment, which was also used to verify pregnancy. The rabbits were euthanized by overdose of Zoletil (Virbac) and samples of the jejunum, liver, kidney, cerebral cortex, and placenta were taken for the study. Pgp content in the samples was measured by ELISA (ELISA kit BlueGene). Tissue samples were homogenized in a DIAX 900 homogenizer at 26,000 rpm in cold PBS (pH 7.2; 1:1 tissue–buffer ratio) for 1 min at 4-6°C, then subjected to three freeze/thawing at -20°C to destroy cytoplasmic membranes, as recommended in the instructions for the kit, and then centrifuged at 1500g for 15 min. The supernatant was used for analysis. Pgp content was recalculated for the total protein mass, which was determined by the Bradford method (Coomassie Plus (Bradford) Assay Kit).

In series II, the rabbits received an injection of fexofenadine, a marker substrate of Pgp, in a dose of 5.5 mg/kg into the marginal ear vein at the indicated times once [5]. In view of the absence of fexofenadine dosage form for parenteral administration, the drug was extracted from Allegra tablets (180 mg, Sanofy). One tablet was crushed and suspended in 20 ml of Acetonitrile (Acros Organics), after which the suspension was shaken on a Shaker for 15 min, followed by centrifugation for 15 min at 3500g. The supernatant was evaporated on a rotary vacuum evaporator, the dry residue was dissolved in 9 ml of water for injection containing 1 ml DMSO [6]. The resultant solution was filtered through a bacterial filter with a pore diameter of 0.22 μm (Corning). The fexofenadine concentration in the solution was determined by HPLC with UV detection. The resulting solution was injected into the marginal ear vein in a volume of 1.1 ml/kg.

After 60 min the animals were sacrificed by Zoletil overdose and liver samples were taken from females and fetuses to determine fexofenadine concentrations by HPLC. The study was carried out on a Stayer chromatograph (Akvilon) with a UV spectrophotometric detector (λ=220 nm) using a reverse-phase chromatographic column Luna C18 100Å (250×4.6) with a grain size of 5 μm at a temperature of 45°C according to the earlier developed method [7].

The obtained results were processed using Statistica 7.0 software (StatSoft, Inc.); the type of data distribution was determined by the Shapiro–Wilk test. In case of normal data distribution, the significance of differences was assessed using the ANOVA test, pairwise comparisons were performed using the Fisher's test. When the data distribution differed from normal, the differences between the series were assessed using the Kruskal–Wallis test. At a significance level of less than 0.05 the parameters were compared in pairs using the Mann–Whitney test with the Bonferroni correction. In case of normal data distribution, the results are presented as $M \pm SD$, otherwise as Me (Q1; Q3).

RESULTS

Progesterone concentration in rabbit serum (Table 1) was increased (p<0.05) on day 7 of pregnancy by 988.1%, on day 14 by 962.3%, on day 21 by 1006.3%, and on day 28 – by 378% in comparison with the control. At the same time the levels of other hormones (estradiol and testosterone) in the blood serum did not significantly differ from those before pregnancy.

It was found that Pgp level in the jejunum and placenta of pregnant rabbits was manyfold surpassed its content in the liver, kidneys, and cerebral cortex (Table 2). The content of the transporter in the jejunum was higher (p<0.05) by 411.2, 309.9, and 228.9% on day 7, 14, and 21 in comparison with that in non-pregnant females. In the liver, Pgp level on day 7 surpassed the control by 46.5% (p<0.05); on day 14 this parameter only tended to increase (p=0.07). In the kidney and cerebral cortex, Pgp content on day 28 of pregnancy was elevated (p<0.05) by 68.5% and 314.2%, respectively, in comparison with non-pregnant animals.

It is known that sex hormones modulate the synthesis and functioning of Pgp [8]. A direct correlation was found between the expression of *mdr1b* gene mRNA encoding Pgp in the placenta and progesterone content in the blood of pregnant mice [9]. A dose-dependent inducing effect of progesterone administered to ovariectomized Chinchilla rabbits on functional activity of Pgp was reported [10]. In our experiment, only the progesterone level significantly changed during all stages of pregnancy; therefore, the increase in Pgp level in the studied organs is probably due to the stimulating effect of this hormone on the transporter synthesis, which is consistent with the results of other *in vivo* studies. The increase in the Pgp level can be due to the effect of the hormone

TABLE 1. Serum Concentrations of Hormones in Female Rabbits before and during Pregnancy

Study period		Estradiol, pg/ml	Progesterone, ng/ml	Testosterone, nmol/liter	Prolactin, mU/ml
Before pregnancy	day 7	323.46±122.50	0.46±0.23	1.176±0.52	21.16±4.30
	day 14	323.70±122.39	0.77±0.38	1.13±0.59	22.50±4.70
	day 21	267.50±41.60	0.63±0.35	0.76±0.12	23.00±6.10
	day 28	269.40±47.92	0.73±0.21	0.89±0.31	35.10±14.90
Pregnancy	gestation day 7	337.67±139.30	4.55±0.77*	1.22±0.35	17.26±3.20
	gestation day 14	304.89±82.93	8.18±1.63*	1.17±0.48	20.30±2.65
	gestation day 21	249.35±54.10	6.97±1.04*	0.94±0.37	23.10±4.90
	gestation day 28	192.10±118.69	3.49±1.63*	0.81±0.32	71.53±7.76*

Note. $p<0.05$ in comparison with the control (ANOVA for independent samples, post hoc Fisher's test).

TABLE 2. Pgp Content (pg/g) in the Tissues of Female Rabbits at Different Stages of Pregnancy (Me (Q1; Q3); *M*±*m*)

Group		Jejunum	Liver	Kidney	Cerebral cortex	Placenta
Control		2226.6 (1899.7; 2536.6)	261.8±71.2	358.7±158.5	310.5±75.4	—
Pregnancy	gestation day 7	11,382.5 (5066.3; 77,233.6)*	383.6±61.3*	279.7±70.4	235.0±67.1	—
	gestation day 14	9127.6 (7241.5; 109,848.4)*	355.8±90.7	328.4±108.4	191.7±85.4	5075.1±1086.6
	gestation day 21	7323.7 (4964.3; 31,314.9)*	161.8±80.8	276.0±52.3	268.5±77.1	1650.4±775.8^{+}
	gestation day 28	2094.2 (1638.8; 2353.2)	374.9±180.7	604.7±159.2*	1286.4±374.5*	2771.3±483.3^{+}

Note. $p<0.05$ in comparison with *control, $^{+}$gestation day 14 (ANOVA for independent samples, post hoc analysis using Fisher's test in case of normal data distribution; Kruskal—Wallis test, pairwise comparison using the Mann—Whitney test with Bonferroni correction for the data distribution other than normal).

on the expression of the *MDR1* gene encoding the ABCB1 protein, interaction with specific progesterone receptors or with transcription factors such as the pregnane X receptor (PXR) or the constitutive androstane receptor (CAR) [11,12].

Analysis of Pgp content in the placenta revealed a significant ($p<0.05$) decrease in this parameter on days 21 and 28 of pregnancy by 67.4 and 45.3%, respectively, in comparison with day 14 of pregnancy.

It is known that the increase in Pgp level does not always correlate with the increase in its activity [2]. Therefore, Pgp activity in the placental barrier was additionally assessed by the degree of penetration of its marker substrate, fexofenadine, from the maternal blood to the fetus. To this end, fexofenadine concentrations in the maternal and fetal liver were compared. The pharmacokinetics of the marker substrate is primarily controlled by Pgp. Fexofenadine does not undergo biotransformation and 90% of the substrate is excreted with the bile, so it is concentrated in the liver at the elimination stage, and is excreted into the bile by Pgp located on the hepatocyte biliary membrane [4].

It was found that fexofenadine concentration in the liver of females remained unchanged throughout pregnancy and was manyfold higher than its content in the fetal liver, which indicated a low permeability of the placenta for fexofenadine and high Pgp activity in the tissue (Table 3). The level of fexofenadine in the fetal liver on days 21 and 28 was significantly ($p<0.05$) higher than on day 14 of pregnancy by 235.7 and 151.7%, respectively, which attested to increased fexofenadine penetration through the placental barrier, and, consequently, reduced activity of the studied transporter protein. Thus, a gradual decrease in the content of Pgp in the rabbit placenta with increasing

TABLE 3. Concentrations of Fexofenadine in the Maternal and Fetal Liver at Different Terms of Pregnancy

Group		Concentration of fexofenadine, μg/g of tissue
Control		15.4±3.56
Maternal liver	gestation day 14	14.7±3.2
	gestation day 21	13.76±2.29
	gestation day 28	12.2±4.2
Fetal liver	gestation day 14	0.28±0.14
	gestation day 21	0.94±0.39*
	gestation day 28	0.72±0.26*

Note. *$p<0.05$ in comparison with gestation day 14 (ANOVA for independent samples, post hoc Fisher's test).

the gestational age is accompanied by a decrease in Pgp activity.

The placenta of rodents and primates, including humans, has a similar hemochorial structure, but Pgp synthesis and activity in the placenta during pregnancy are species-specific. In a study on mice (wild-type FVB), Pgp was detected in the placenta by Western blotting; its level increased starting from gestation day 10, but decreased by the end of pregnancy [13]. In female macaques, Pgp activity in the placenta and the blood–brain barrier assessed by using Pgp substrate verapamil increased with increasing gestational age [14]. In humans, immunohistochemistry and Western blotting revealed the presence of Pgp in the placental barrier during the first trimester of pregnancy, however, with an increase in gestational age, starting from week 13-14, the transporter level in the chorionic villi sharply decreased [15] (Mathias A.A., *et al.*, 2005).

Our study showed a decrease in Pgp content and activity in the placenta in rabbits with increasing gestational age, which is comparable with the dynamics of Pgp functioning in humans.

Thus, it was found that Pgp content in the jejunum and placenta of rabbits manyfold surpassed that in the liver, kidneys, and cerebral cortex. An increase in Pgp content in comparison with non-pregnant females was revealed in the jejunum on days 7, 14, 21, and 28 of pregnancy, in the liver on day 7 with a tendency to increase on day 14, in the kidney and cerebral cortex on day 28, which is probably due to an increase in the serum concentration of progesterone. With increasing gestational age, a decrease in Pgp content and functional activity in the placental barrier was noted, which was confirmed by an increase in the penetration of Pgp marker substrate fexofenadine through the barrier.

REFERENCES

1. Mollazadeh S, Sahebkar A, Hadizadeh F, Behravan J, Arabzadeh S. Structural and functional aspects of P-glycoprotein and its inhibitors. Life Sci. 2018;214:118-123. doi: 10.1016/j.lfs.2018.10.048
2. Yakusheva EN, Titov DS. Structure and Function of Multidrug Resistance Protein 1. Biochemistry (Mosc). 2018;83(8):907-929. doi: 10.1134/S0006297918080047
3. Ceckova-Novotna M, Pavek P, Staud F. P-glycoprotein in the placenta: expression, localization, regulation and function. Reprod. Toxicol. 2006;22(3):400-410. doi: 10.1016/j.reprotox.2006.01.007
4. Yakusheva EN, Sychev DA, Shchul'kin AV, Chernykh IV, Gatsanoga MV. In vivo assessment of drugs belonging to inhibitors and inductors of P-glycoprotein. Exp. Clin. Pharmacol. 2018;81(1):17-23. doi: 10.30906/0869-2092-2018-81-1-17-23
5. Manual for Preclinical Studies of New Pharmacological Substances. Mironov AN, ed. Moscow, 2013. Russian.
6. Chernykh IV, Shchul'kin AV, Myl'nikov PYu, Gatsanoga MV, Popova NM, Yakusheva EN. A Method of Analysis of the Functional Activity of P-Glycoprotein in the Blood-Brain Barrier. Neurochem. J. 2019;13(1):105-109. doi: 10.1134/S1819712419010069
7. Mylnikov PYu, Chernykh IV, Shchulkin AV, Popova NM, Yakusheva EN. HPLC methods of fexofenadine quantitative analysis in rabbits' liver. Pharm. Pharmacol. 2020;8(1):40-47. doi: 10.19163/2307-9266-2020-8-1-40-47
8. Erokhina PD, Abalenikhina YuV, Shchulkin AV, Chernykh IV, Kotlyarova AA, Pravkin SK, Slepnev AA, Yakusheva EN. Study of influence of estradiol on the activity of P-glycoprotein in vitro. Nauka Molodykh (Eruditio Juvenium). 2020;8(3):329-336. Russian. doi: 10.23888/HMJ202083329-336
9. Mathias AA, Hitti J, Unadkat JD. P-glycoprotein and breast cancer resistance protein expression in human placentae of various gestational ages. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2005;289(4):R963-R969. doi: 10.1152/ajpregu.00173.2005
10. Shchul'kin AV, Chernykh IV, Yakusheva EN, Popova NM, Myl'nikov PY. Experimental studies of the effects of progesterone on the functional activity of P-glycoprotein. Pharmaceut. Chem. J. 2018;52(7):587-592. doi: 10.1007/s11094-018-1864-8
11. Coles LD, Lee IJ, Voulalas PJ, Eddington ND. Estradiol and progesterone-mediated regulation of P-gp in P-gp overexpressing cells (NCI-ADR-RES) and placental cells (JAR). Mol. Pharm. 2009;6(6):1816-1825. doi: 10.1021/mp900077q
12. Kliewer SA, Moore JT, Wade L, Staudinger JL, Watson MA, Jones SA, McKee DD, Oliver BB, Willson TM, Zetterström RH, Perlmann T, Lehmann JM. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell. 1998;92(1):73-82. doi: 10.1016/s0092-8674(00)80900-9
13. Zhang H, Wu X, Wang H, Mikheev AM, Mao Q, Unadkat JD. Effect of pregnancy on cytochrome P450 3a and P-glycoprotein expression and activity in the mouse: mechanisms, tissue specificity, and time course. Mol. Pharmacol. 2008;74(3):714-723. doi: 10.1124/mol.107.043851
14. Chung FS, Eyal S, Muzi M, Link JM, Mankoff DA, Kaddoumi A, O'Sullivan F, Hsiao P, Unadkat JD. Positron emission tomography imaging of tissue P-glycoprotein activity during pregnancy in the non-human primate. Br. J. Pharmacol. 2010;159(2):394-404. doi: 10.1111/j.1476-5381.2009.00538.x
15. Gil S, Saura R, Forestier F, Farinotti R. P-glycoprotein expression of the human placenta during pregnancy. Placenta. 2005;26(2-3):268-70. doi: 10.1016/j.placenta.2004.05.013