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Active and passive transport of drugs in the human placenta

Czynny i bierny transport leków w łożysku ludzkim

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Abstract

The human placenta, characterized by the processes of passive transport and facilitated diffusion, contains numerous active transport proteins, usually located in the microvilli of the syncytiotrophoblast or in the endothelium of the capillaries of the villi. These proteins use either the energy from ATP hydrolysis or other mechanisms resulting, among others, from the formation of the maternofetal ion gradient, which facilitates the transfer of various endogenous substances or xenobiotics across the body membranes. The proteins either trigger the efflux of these substances from the fetal tissues via the placenta into the maternal bloodstream, or conversely, they accumulate them in the fetal tissues. Both the placenta and the fetus are equipped with independent systems of enzymes of 1st and 2nd phase of substrate metabolism, such as CYP450, glucuronyltransferase or sulphatase.

An active therapy with a wide range of drugs, often at high toxicity levels, either shortly before or during pregnancy, has naturally posed a question concerning the degree of impermeability of the placental barrier and how effectively it can be crossed, including any possible negative embryotoxic or teratogenic consequences. Such hazards seem to be quite real, as many drugs are substrates for ABC transporters. Also the placenta itself, including its structure, is subject to vast transformations during pregnancy, which may be observed as the thinning of the barrier separating the maternal blood from the fetal one, from 20-30μm in the first trimester of gestation down to 2-4μm in the third trimester of gestation.

Key words: **placenta** / **drug transport** / **multidrug-resistance protein** / / **pregnancy** / **syncytiotrophoblast** /

Streszczenie

W łożysku ludzi oprócz transportu biernego oraz dyfuzji ułatwionej zawartych jest wiele aktywnych białek transportowych zazwyczaj zlokalizowanych w mikrokosmkach syncytiotrofoblastu lub w śródbłonkach naczyń włosowatych kosmków. Wykorzystują energię z rozpadu ATP lub inne mechanizmy wynikające między innymi z formułującego się gradientu jonów pomiędzy płodem i matką sprzyjają przenikaniu przez błony różnorodnych substancji endogennych lub ksenobiotyków. Kreuja albo ich wyrzut z tkanek płodu przez łożysko do krwi matki lub wręcz przeciwnie kumulują je w jego tkankach. Samo łożysko i płód dysponuje samodzielnymi układami enzymatycznymi pierwszej i drugiej fazy przemian substratów takimi jak: CYP450, glukuronylotransferazy czy sulafatazy.

Aktywne postępowanie terapeutyczne z wykorzystaniem szerokiej gamy leków niejednokrotnie o wysokim poziomie toksyczności w okresie przed ciążowym oraz w ciąży rodzi pytanie na ile bariera łożyskowa jest szczelna oraz na ile jest skutecznie pokonywana z możliwymi negatywnymi konsekwencjami o charakterze embriotoksycznym lub teratogennym. Groźba wydaje się być całkowicie realna, bowiem wiele leków to substraty dla transporterów ABC. Również samo łożysko i jego struktura ulega w trakcie ciąży głębokim przewartościowaniom czego miarą może być redukcja dzielącej barierę krew matki od krwi płodu z 20-30μm w pierwszym trymestrze ciąży do 2-4μm w trzecim trymestrze ciąży.

Słowa kluczowe: **łożysko** / **transport leków** / **białko oporności wielolekowej** / / **ciąża** / **syncytiotrofoblast**

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Introduction

Over the last several decades, the consumption of medicines either shortly before or during pregnancy has been increasing. It can be attributed to both the medical progress and the widely understood civilization changes [1]. The US data demonstrate that two thirds of women at the above-mentioned life stages take various medicines, often characterized by a high-toxicity level [2]. Among women who conceive and give birth are those with the following diseases: breast, thyroid, cervix and ovarian cancers, and Hodgkin disease [3]. Pregnancy also occurs among female patients undergoing regular treatment for such disorders as diabetes, circulatory problems, epilepsy, psychiatric disorders, and those who are HIV-positive [4]. According to the recent estimates, the number of the last-mentioned females approaches to 18-19 million in the world [5]. Also pregnancy itself, including its pathological course, has become an object of medical intervention. The number of diagnostic procedures aimed at the fetus, prognosticating its developmental or genetic defects, etc., has been constantly growing. As a result, pregnancy, the embryo, and finally the fetus, often become an object of a voluntary or involuntary intensive therapeutic management involving the use of drugs that are characterized by high levels of direct toxic effect or by long-term effects that are too difficult to predict. Active participation of women in the job market and their successful competition at work with men, have also resulted in an increased drug consumption among them, often without medical consultation and solely due to the pressure of aggressive advertisements in the media. The drugs whose consumption by women shortly before or/and during pregnancy has considerably increased, include: hypnotics, tranquillizers, painkillers, mood-improving drugs, diuretics, anti-emetics, and others [6].

In this situation, the knowledge of the role of the hemochorial placenta becomes crucial, as it is not only involved in supplying the fetus with oxygen, nutrients, cytokines, growth factors, hormones, and in eliminating toxic metabolites, but also acting as a protective barrier for endobiotics, xenobiotics, systems of passive drug transport, facilitated diffusion, and active transport, the last –mentioned one represented, among others, by ABC transporters with an ATP-binding cassette. The knowledge of these issues is indispensable to draw rational and effective therapeutic decisions concerning pregnancy and, simultaneously, to minimize any possible hazards to human health and fetal development. The issue is further complicated by the fact that in the course of pregnancy the placenta not only undergoes significant structural transformations but also participates in the bio-transformation of xenobiotics and endogenous substrates, to a varying extent related to the particular trimester of gestation. These processes, in turn, are subject to modifications effected by smoking and alcohol, psychoactive substances intake [7, 8].

In Poland, approximately 40% of women smoke during pregnancy. The placental transport systems, both active and passive, mutually compete for the substrates, and often their transport vector changes. Besides, in the course of pregnancy, the expression of the active transporters in the particular cells and structures of the placenta evolves. Some of them dominate in the first trimester of gestation, others gradually increase their activity as the organ matures, yet others are characterized by a stable action throughout the whole gestational period [9, 10].

The advancement of the processes of exchange of various substrates is related to:

a. Increasing contact surface of the placental syncytiotrophoblast with the maternal blood from 3.4m^2 at 28 weeks of gestation up to 12.6m^2 in the third trimester.
b. Increasing intensity of blood flow in the placental vessels from 50ml per minute at 10 weeks of gestation up to 600 ml per minute at the termination of pregnancy.
c. The thinning /decreasing diameter/μm of the barrier separating the maternal blood from the fetal one from 20/30μm at the beginning of pregnancy down to 4μm at term pregnancy [11].

The aim of this article is to outline these issues to obstetricians, perinatologists and other specialists.

The placental structure

Human beings have at their disposal the hemochorial placenta, characterized by a direct contact of the developing fetal tissues with the maternal blood. This is a discoid, invasive placenta. It can be distinguished from the endotheliochorial placenta, found in dogs and cats, and also, from the epitheliochorial placenta, typical for sheep, swine, or horse. (Table I).

Its structure is composed of the fetal tissue groups, forming the chorionic membrane along with the villi, and the maternal part, the so-called decidua /deciduous membrane/. Each placenta forms from twenty to forty well-ordered compartments called cotyledons [11]. The latter function as an open vascular system. Each of the cotyledons fills up the chorionic tree, which is the consequence of the ramification of the chorionic trunks into the so-called terminal villi, the tertiary ones. Some of the branches of the chorionic trunk reach the basal deciduous membrane as the so-called anchoring villi. Thus, each chorionic trunk, with its numerous terminal villi, is separated from the fetal part by the chorionic membrane, from the maternal part by the deciduous membrane, and from the sides, by the incomplete deciduous septa. The system of the barriers between the maternal blood and the fetal blood is formed by the continuous endothelium of the fetal capillary circulation, the mesenchymal tissue with Hofbauer cells (forming the stroma of the villi), the syncytiotrophoblast resting on the continuous basal membrane, and the underlying cytotrophoblast. It is the syncytiotrophoblast which plays a particularly important role in the formation of the above-mentioned barrier and also in the formation of the active transporters of various xeno- and endogenous substrates. In its free /unbound/ surface facing the maternal blood, the syncytiotrophoblast is equipped with a vast system of micro-villi, considerably increasing the substrate-exchange surface. At the initial stages of placental development, and in particular, in the first trimester of pregnancy, the epithelium is stratified, as a rule, and the thickness, or diameter, of the barrier often exceeds 20-30μm. In the course of pregnancy, as the fetal weight increases, the placental barrier undergoes gradual thinning so that towards the final phase of gestation (at term pregnancy) it is reduced down to 2-5μm in diameter [8, 11]. The above-mentioned reduction in the barrier thickness is due to the extension of the chorionic villi capillaries, their shift to the vicinity of the syncytiotrophoblast basal membrane, atrophy or considerable reduction of, and the continuous arrangement of, the cytotrophoblast cells, and considerable transformations in

the structure of the polynucleous syncytiotrophoblast cells, consisting in the formation of the vasculo-epithelial membranes and the syncytiotrophoblastic buds. The above-mentioned process of placental structure transformation is accompanied by vast changes in the activities of its drug-metabolizing systems, including the 1st and 2nd phase enzymes, as well as by the quantitative and qualitative evolution in the particular transporters [8, 11]. As a result of the above-signaled transformations within the placental structure and due to changes in the activity of the enzymes metabolizing the exo- and endogenous products as well as changes in the expression of the various transporters from ABC family or other transporters, the barrier role of the placenta undergoes considerable evolution in the course of pregnancy: from the prevalence of the protective function in the initial phase of pregnancy, i.e. when the embryo (and then, the fetus), forms its blastodermic layers, to the favor of other functions, including the metabolic ones, resulting from the ever-increasing fetal demand for oxygen, nutrients, hormones, and other products [4, 11].

Unfortunately, lack of the fetomaternal circulation up to 10 weeks of gestation, i.e. in the phase of an intensive organogenesis of the embryo, does not completely eliminate the toxic or embryotoxic effects of xenobiotics, which effectively influx the fetal cells by way of the bodily tissue fluid via the wide intercellular spaces.

The mechanisms of drug transportation in the placenta

In addition to the earlier-mentioned active transporters from ABC family, the placental exchange is effected in other ways as well, including passive transport, facilitated diffusion, phagocytosis and pinocytosis. The latter two ways are of secondary clinical significance, as they are hardly effective. For instance, glucocorticoids, ganciclovir and cephalosporin can be transported in this way [11].

Passive transport

Undoubtedly, passive transport appears to be the main way of substrate exchange in the placenta. It uses the differences in the concentrations of the substances transported between the maternal blood and the fetal tissues. The difference in the concentration gradient of these substances triggers their efflux.

The physical diffusion does not require energy input, the process does not undergo saturation or competence inhibition. Drugs undergoing this process are those characterized by a low molecular weight, good solubility in fats, and they are usually non-ionized. Drugs with the molecular weight of 500 daltons permeate though the placenta only in part, and those weighing above 1000 daltons do this with extreme difficulty [11]. The course and effectiveness of the passive diffusion undergo modifications which are subject to the conditions developed by the placenta itself, by its structure, and dependent on the biochemical and physiological processes occurring within the placental area. The placental membranes are easily permeable to drugs soluble in lipids, but hardly permeable to drugs soluble in water [11]. Most drugs, being weak acids or alkali at the physiological pH, are found in the ionized form, which inhibits their passage towards both the mother and the fetus. Drugs that circulate freely, unbound to blood proteins, permeate through the placento-fetal barrier. Although many of these proteins may temporarily bind to drugs, the leading role is given to albumins and α1 acidous glycoprotein. Albumins bind to drugs that are acidous, whereas α1. glycoprotein binds to alkaline drugs. As the gestational age increases, the content of the acidous glycoprotein in the maternal bloodstream remains on a stable level, whereas the albumin content systematically decreases. Conversely, α1 glycoprotein content increases from approximately 10% of the level found in the maternal blood at 10 weeks of gestation to 30-40% at term pregnancy. Therefore, in consequence of the above-described evolution in the concentrations of the acidous glycoprotein in the fetal blood, one may prognosticate increased concentration values of the alkaline drugs in the fetal tissues. Furthermore, the affinity of the above-mentioned proteins to certain drugs undergoes changes. Albumins are characterized, among others, by high affinity to such drugs as: local anesthetics, phenobarbital, phenytoin, and by lower affinity to salicylates. A number of drugs become periodically deposited within the area of the placental structures. Such is the case with buprenorphin [11].

Table I. Structural and functional features of selected mammalian placentas.

Species	Tissue layers between maternal and fetal circulations	Placental structure	Flow pattern
human	hemomonochorial	villous	multivillous
Rhesus monkey	hemomonochorial	villous	multivillous
Guinea pig	hemomonochorial	labyrinthine	countercurrent
rabbit	hemodichorial	labyrinthine	countercurrent
rat	hemotrichorial	labyrinthine	countercurrent
mouse	hemotrichorial	labyrinthine	countercurrent
cat	endotheliochorial	lamellar	crosscurrent
pig	epitheliochorial	labyrinthine	double crosscurrent
sheep	epitheliochorial	folded	multivillous to countercurrent

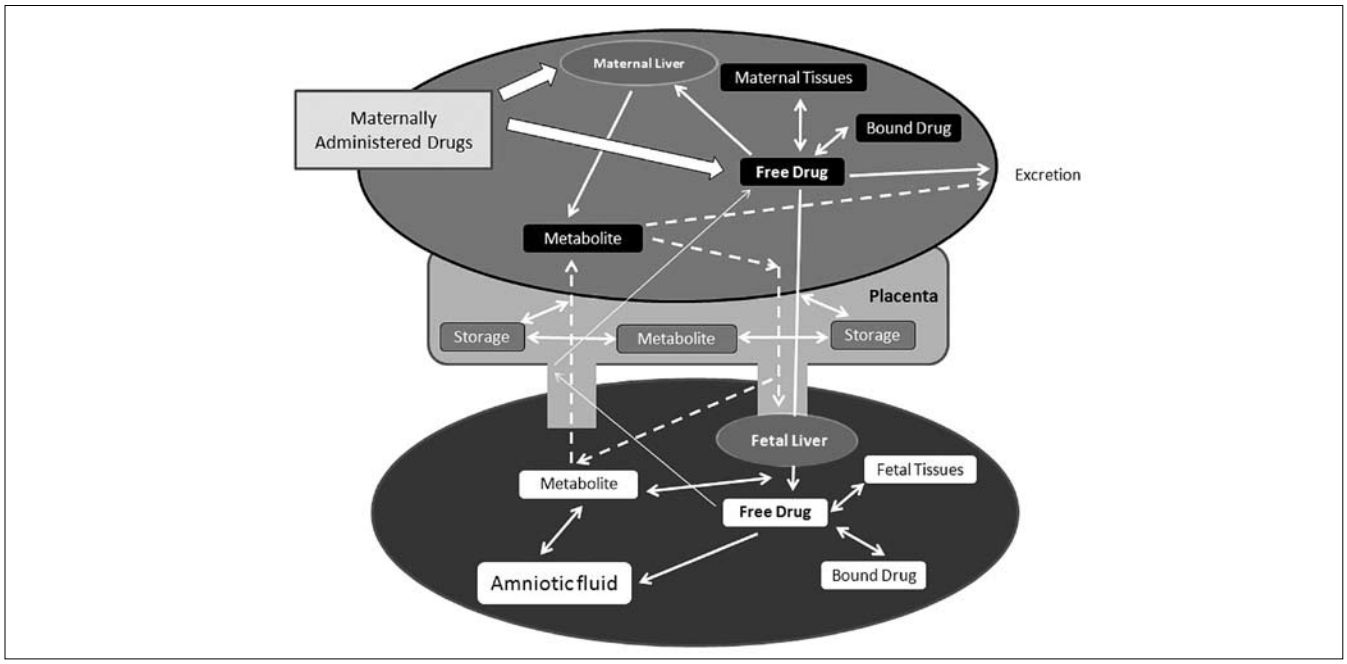

Figure 1. Schematic representation of bidirectional transport of drugs and their metabolites across the human placenta.

Active transport

The proteins playing a fundamental role in active efflux of xenobiotics and endogenous substrates of the syncytiotrophoblast into the maternal blood include transporters with the ATP-binding cassette (ABC family). These proteins are characterized by a considerable homology of the amino-acid sequence. They use the energy produced as a result of ATP hydrolysis for the transportation of many substrates, in defiance of the concentration gradient [6]. The molecules of the above-mentioned transporters have 12-17 transmembrane regions. Most of them act in the form of monomers as the so-called "semi-transporters", and some of them dimerize e.g. breast cancer resistance protein –BCRP [12]. What seems worth considering, is the vast diversity of the transported substrates, both exo- and endogenous. This diversity makes the following question how these transporters ensure adequate transport-specificity? [12, 13] relevant.

The effective drug efflux pumps, effluxing drugs from the fetal blood and from the syncytiotrophoblast cells into the maternal blood, include: immunosuppressive drugs, HIV protease inhibitors, antineoplastic drugs, tranquillizers, anticonvulsants, antiparasitic and antibacterial drugs, cardiovascular drugs, and others [12]. Among the effluxed endogenous substrates are: steroid hormones, thyroxin, certain cytokines, vitamins, prostaglandins [12]. The suprafamily of ABC transporters having the ATP-binding cassette includes: P-glycoprotein (P-gp), MDR 1/ABCB 1 and MDR 3/ABCB 4, breast cancer resistance protein (BCRP/ABCG 2), and multidrug resistance proteins (MRPs /ABCC 1-6 and 10-11) [4].

P-glycoprotein (MDR 1/ ABCC 1)

It is one of the most important and earliest-known transporters. This relatively large protein (170kD), is located exclusively on the apical surfaces of the microvilli, which significantly extend the substrate area within the syncytiotrophoblast (Fig. 2).

It transports hydrophobic, uncharged, weak alkaline substrates whose molecular weight varies from 220-1880 Da., in the fetomaternal direction [11]. It participates in the elimination of such toxic metabolites, forming within the placental area and other organ regions, as: unconjugated bilirubin, free fatty acids, neurotransmitters, estradiol conjugated with glucuronic acid, and glycocorticoids. Drugs that undergo elimination include: verapamil, nifedipine, digoxin, paclitaxel, etoposide, vinblastine, doxorubicin, methadone, phenytoin, simvastatin, and many others. The experimental research on mice with mdr1 knock-out gene and on those deprived of glycoprotein (P-gp) has convincingly demonstrated that the above-mentioned transporter has a significant fetoprotective function, protecting the fetus against the toxic or teratogenic activities of many xenobiotics and their indirect metabolites [11].

In turn, the product of MDR 3 /ABCB 4 gene acts in the hepatic cells as a lipase in the translocation of phospholipids to the bile [14, 15].

Structurally, it is located in the endoplasmic reticulum, i.e. in the close vicinity of the enzymes from the cytochromeP450-dependent monooxygenase group, converting xenobiotics. In the placenta, the expression of MDR 3 has been demonstrated in the baso-lateral segments of the syncytiotrophoblast plasmalemma and in the endothelial cells of the capillaries of the villi. Its activity increases in the course of gestation and reaches its peak in the third gestational trimester, which distinguishes it significantly from Pgp1, which dominates in the first trimester [10].

The transport protein under discussion not only participates in the transport of phospholidylothoine but also in the transport of the bile acids, digoxin, vinblastine, and paclitaxel. The transportation role of the MDR 3 protein in the placenta is not very well documented.

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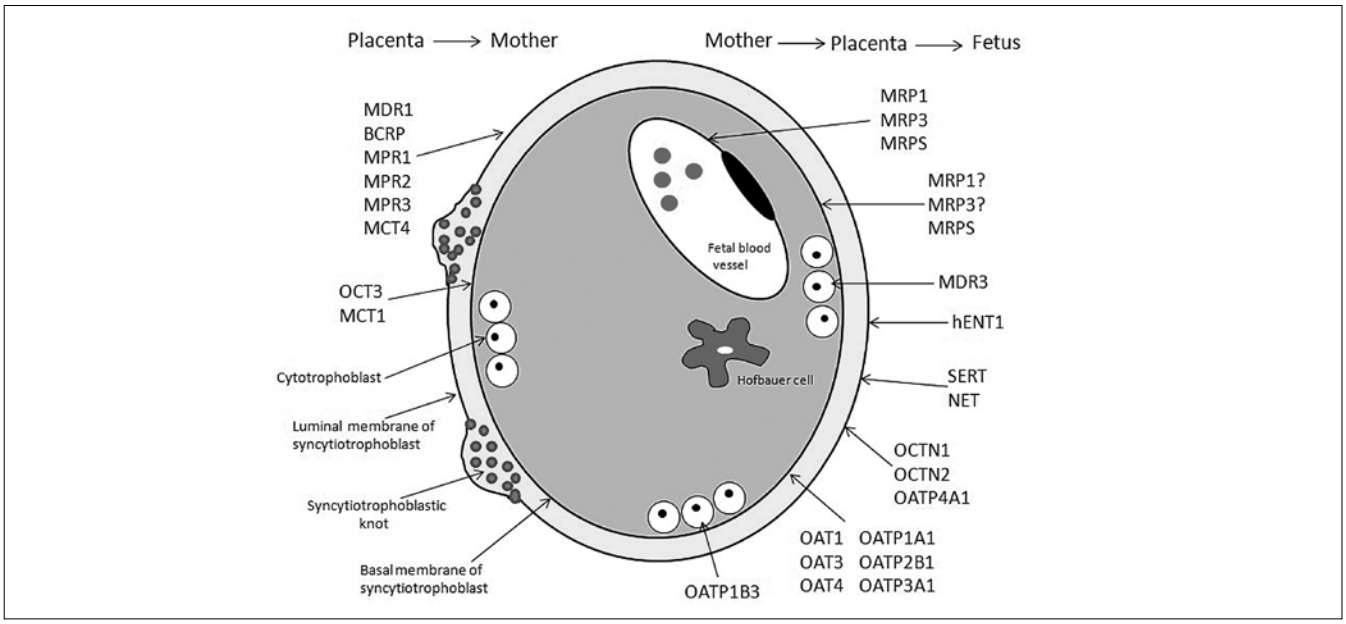

Figure 2. Histological localization of drug transporters in the terminal villus of the human placenta.
BCRP: breast cancer-resistance protein; hENT: human equilibrative nucleoside transporter; MCT: monocarboxylate transporter, MDR: multi-drug resistance, MRP: multi-drug resistance protein; NET; norepinephrine transporter, OAT: organic anion transporter; OCT: organic cation transporter; OCTN organic cation/carnitine transporter; SERT: serotonin transporter (see text for detailed information).

MRP/ABCC multidrug resistance proteins

The molecular weight of these proteins varies from 190-200 kD. They participate in the process of ATP-dependent transmission of the endogenous amphiphilic and lipophilic substrates and xenobiotics, conjugated with sulphates or glucuronic acid. These proteins with additional 250 aminoacids in the molecule are characterized by a higher substrate specificity than Pgp in relation to many drugs. MPR1, MPR4, and MPR5 are widely represented in various organs, whereas MPR2, MPR3, and MPR6 are usually located in the liver, kidneys and intestines [4].

Drugs that are effluxed from the fetal blood into the maternal blood, or in the opposite direction, include: methotrexate, etoposide, vinblastine, vincristine, HIV-protease inhibitors, some antibiotics from the fluorochinolons group, ampicillin. They show a diversified cellular location in the placenta and different expression levels in the particular gestational trimesters [4].

MPR1 expression increases with maturation of the placenta, and, immunocytochemically, it is located in the endothelial cells of the capillaries of the villi and at the base of the syncytiotrophoblast [16].

The activity of MPR2, in turn, is restricted solely to the microvilli of the syncytiotrophoblast and is also dominant in the late gestational phase. As far as MPR3 is concerned, it shows a stable activity level until the end of the third gestational trimester. It is found both in the syncytiotrophoblast and in the endothelia of the capillaries of the villi. Some data in the literature seem to suggest that this pump propels medicines into both the fetal and the maternal organisms [17].

As regards MPR4, although it shows transport activity in the placenta towards many substrates, yet, there is no certainty as to in what structures it is located. It effluxes zidovudine from the placenta into the maternal blood, it transports several prostaglandins including PGE1 and PGE2, playing an important role in the regulation of the vascular bed of the villi and in other mechanisms regulating the homeostasis of the fetus [19].

MPR5 transports in the placenta the cyclic nucleotides, particularly Comp, locating itself in the vesicles and vacuoles that are distributed in the close vicinity of the basal membrane of the syncytiotrophoblast and in the endothelia of the capillaries of the villi. Its increased activity pertains to the first trimester of gestation, in which it regulates the organogenesis and differentiation of the fetus by affecting the Comp level.

As to MPR7/ABCC10, MPR8ABC11 and ABCC13, they are found in the placental cells in low concentrations. Their cellular location has not been established yet. They probably play a role in fetal resistance to some antiviral drugs. They efflux from the placenta into the maternal blood such substances as: cyclic nucleotides, bile acids, conjugated steroids, eicosanoids [19].

Breast cancer resistance protein (BRCP/ABCG2)

It is also known as mitoxantrone-resistance protein or ABC placental specific protein [20, 21]. It is characterized by high mRNA and protein levels throughout the whole gestational period. It is located in the microvilli of the syncytiotrophoblast of the villi and in the uterus [21].

Although the role of the above-discussed transporter in the placenta has not been ultimately established, there are many arguments in favor of its status as an effective and stable partner for the placental barrier, in addition to P-gp, in respect to such substrates as: organic cations, lipophilic conjugated derivatives, and in particular, antineoplastic drugs (topotecan, mitoxantrone, quercetin, doxorubicin, methotrexate) [22]. It also effluxes from the placenta and the fetus into the maternal blood such substances as imatinib, nitrofurantoin, and zidovudine. Besides, it removes the conjugated steroids.

Placental transporters of SLC soluble substrates

Organic anion transporters (OAT/SLC22A family)

This family plays a dominant role in the elimination through the kidneys of a number of metabolites such as urates, uremic toxins, fatty acids, prostaglandins, conjugated steroids, neurotransmitter metabolites, and selected xenobiotics (chemotherapeutics, β-lactam antibiotics, and antiviral nucleoside analogues [23]. In the placenta, the presence of OAT1 and OAT3 in the basal membrane, on which the syncytiotrophoblasts rest, and OAT4 in the syncytiotrophoblast cells, has been confirmed. The first two organic anion transporters are connected with the efflux of xenobiotics from the fetal circulation, whereas the third mentioned one effluxes the conjugated steroids into the maternal circulation [4, 25].

Polypeptide organic anion transporters OATP/SLCO

In the placenta, the expressions of OATP A, OATP B, OATP 8, OATP D, and OATP E have been confirmed [25, 26, 27, 28]. OATP B is located in the basolateral segment, whereas OATP A, at the apex of the microvilli of the syncytiotrophoblast covering the villi.

Organic cation transporters SLC22

OCTN1/SLC22A4 and OCTN2/SLC22A5 are located in the microvilli of the syncytiotrophoblast. They demonstrate a high level of activity in the third gestational trimester with its peak just before parturition. OCTN1 transports bile salts and antiviral drugs in the feto-maternal direction, whereas OCTN 2 transports carnitine in the opposite direction [29].

Monoamine transporters SLC6A and SLC22A

SERT/SLC6A4 serotonin transporter and NET/SLC6A2 dopamine-norepinephrine transporter are located in the apical surface of the microvilli of the syncytiotrophoblast. They transport to the fetus, respectively, serotonin and dopamine, and also amphetamine. The effectiveness of this process depends on the established sodium and chloride anion gradient.

The OCT3/SLC22A3 transporter, in turn, whose activity is determined by the current membrane potential, transports in the fetomaternal direction, via the placenta, not only monoamines but also amphetamine, imipramine, clonidine, and cimetidine. Immunocytochemically, it is located in the syncytiotrophoblast plasmalemma, adjacent to the basal membrane.

Carboxylate transporters SLC16

The proton gradient, in the case of monocarboxylates, and the sodium ion gradient, in the case of dicarboxylates, are used as the driving force for the efflux through the placental barrier. NCT1 is located in the basilar membrane, whereas NCT4 on the apical surface of the syncytiotrophoblast. NCT4 removes lactates from the fetus and the placenta [30].

It also transports acetylsalicylic acid and organic acids in accordance with the concentration gradient in the materno-fetal compartment.

Nucleoside transporters SLC29A

hENT1/SLC29A1 and hENT2/SLC29A2 are located in the syncytiotrophoblast cells, in the microvilli and in the basal cell membrane, respectively. hENT3, in turn, shows a structural connection with the intracellular membranes of the syncytiotrophoblast. They are probably lysosomes [31, 32]. The distribution of hENT2 is not yet known.

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