Transport and toxicity of 5-fluorouracil, doxorubicin, and cyclophosphamide in *in vitro* placental barrier model based on BeWo b30 cells

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An *in vitro* placental barrier model based on human choriocarcinoma BeWo b30 cell line was considered as a method of preclinical study of the transport and toxicity of antitumor agents and other organic compounds. Low permeabilities were found for 5-fluorouracil as an example of hydrophilic compound and for doxorubicin as an example of a lipophilic compound with a high degree of binding to proteins and DNA and a high permeability was found for cyclophosphamide as an example of lipophilic compound with a low degree of binding to proteins. Using impedance spectrometry and cell viability assessment *via* reduction of resazurin to resorufin, a pronounced cytotoxic effect of doxorubicin and good tolerance of 5-fluorouracil and cyclophosphamide by the cells were shown for drug concentrations equal to the maximum concentrations in the patients′ blood during the treatment of breast cancer.

Key words: 5-fluorouracil, cyclophosphamide, doxorubicin, breast cancer, placental barrier, BeWo b30, cell model.

Development of new chemical agents for the treatment of cancer and modification of existing agents are important because of increasing occurrence of malignant diseases.[1] It is necessary to perform comprehensive preclinical studies of new molecules before human trials. In particular, it is necessary to predict the possible influence of the drugs on pregnant women and to evaluate the degree of drug penetration through the placenta into the fetal blood circulation.[2] The human placental barrier consists of trophoblast cells, connective tissue structures, including basal membrane, and fetal endothelium.[3,4] The placental barrier permeability of drugs can be predicted using various preclinical models. Among animal models, rodents and primates, which possess, like humans, hemochorial placenta, most closely resemble humans in the placental structure.[5] However, the rodent placenta differs from that of humans both at the anatomical (labyrinthal rather than villus structure) and histological levels (in rodents, the mother and fetus blood circulations are separated by three cell layers, unlike two layers present in humans). Experiments on primates are expensive. In addition, animal cells differ from human cells in the protein structure and other features.[6] The *ex vivo* studies using placentas obtained after the delivery or cesarean section are characterized by high variability of the results and low frequency of success. In addition, the placenta after the delivery is degenerated and poorly reflects the condition of placenta in the second trimester of pregnancy.[7]

The cell lines derived from human tumors can be used for various model experiments *in vitro*.[8] A good alternative for studying the permeability of the placenta to chemicals are *in vitro* models based on human cells.[9,10] In particular, the placental barrier is often modeled using the BeWo b30 cell line, which forms a complete monolayer with tight junctions and creates necessary conditions for studying the barrier properties of placenta.[11,12] Human vascular

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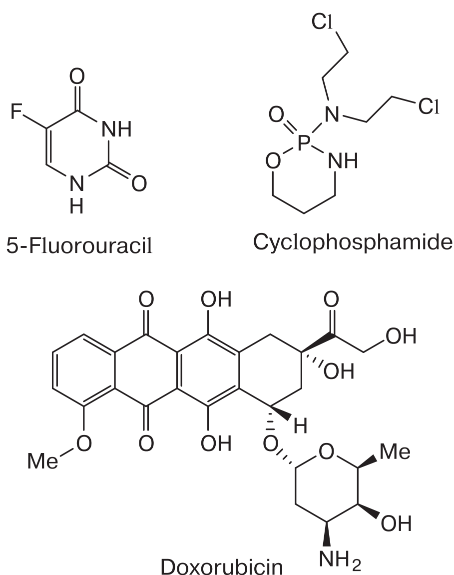
endothelium is often modeled by the primary human umbilical vein endothelial cells (HUVEC).[10,13] This *in vitro* procedure can be used to estimate the permeability of the placental barrier in the development of new drugs and drug modifications.

Breast cancer is the most frequent type of cancer that can occur during pregnancy. Most of the medications are low-molecular-weight lipophilic compounds, which is favorable for their transport through the placenta to the fetal blood. Therefore, it is recommended to start the chemotherapy after the first trimester of pregnancy when the formation of fetal organs has mainly completed.[14] However, even in this case, the medications can retard the fetal growth and lead to premature delivery and low birth weight, which indicates that they do penetrate the placenta. Therefore, study of this process is topical.[15]

The breast cancer in pregnant women is treated, most often, according to the FAC protocol, which includes the use of 5-fluorouracil, doxorubicin (or its stereoisomer, epirubicin), and cyclophosphamide.[16] These three drugs belong to different classes and have special chemical features. 5-Fluorouracil is a hydrophilic derivative of uracil, a pyrimidine base present in the RNA of living organisms; it has molecular weight of 130.1 g mol^{-1} and prevents cell division by blocking DNA synthesis *via* inhibition of thymidylate synthase and by blocking RNA synthesis *via* incorporation into RNA upon transcription.[17] Cyclophosphamide is a lipophilic cytostatic with the molecular weight of 261.1 g mol^{-1} and alkylation type of action, which generates alkyl cross-links in cellular DNA and proteins and has low degree of binding to blood proteins.[18] Doxorubicin is a lipophilic anthracycline cytostatic with the molecular weight of 543.5 g mol^{-1} and high degree of binding to blood proteins and a DNA intercalator preventing DNA replication in the cell, which also promotes the formation of free radicals responsible for cytotoxicity.[19]



Analysis of the physicochemical properties and transport of these drugs of different classes through the placental barrier would shed light on the permeability and effect on the placental cells and reveal the efficiency of the developed procedure for assessment of new drugs.

Thus, the purpose of this work is to study an *in vitro* placental barrier model in order to evaluate the transport and toxicity of chemical compounds for the treatment of tumor diseases.

Experimental

Reagents and materials. The following materials were used: 1X Dulbecco phosphate buffer (DPBS, PanEco, Russia); Gibco DMEM cell culture medium with glucose content of 4.5 g L^{-1} (Thermo Fisher Scientific, USA); Gibco FluoroBrite DMEM cell culture medium without Phenol Red (Thermo Fisher Scientific); Gibco One Shot fetal bovine serum (Thermo Fisher Scientific); L-glutamine (PanEco); a 0.25% trypsin EDTA solution with Hanks′ salts (PanEco); a 100X Gibco MEM NEAA solution of nonessential amino acids (Thermo Fisher Scientific); a 100X Gibco Pen Strep penicillin and streptomycin solution (Thermo Fisher Scientific); a CellTiter-Blue reagent kit for evaluating cell viability (Promega, USA); 5-fluorouracil (Sigma—Aldrich, USA); ISOPAC cyclophosphamide monohydrate (Sigma—Aldrich); doxorubicin hydrochloride (Pharmachemie, the Netherlands); formic acid (Sigma—Aldrich), acetonitrile (Sigma—Aldrich); Corning HTS Transwell 96-well culture systems (Merck, USA).

Cell culturing. The BeWo human choriocarcinoma cell line (b30 clone) was received from Prof. K. Albrecht (University of Bern, Switzerland) as a courtesy of Dr. A. Schwartz (University of Washington in St. Louis, USA). The cells were cultured by a previously described procedure[20] in the Gibco DMEM medium with a glucose content of 4.5 g L^{-1} with the addition of 10% fetal bovine serum, 2 m*M* L-glutamine, 1X solution of nonessential amino acids, and 1X solution of penicillin and streptomycin (100 U mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin).

For permeability experiments, the BeWo b30 cells were added into the inserts of Transwell 96-well supports in a quantity of 10 000 cells per insert (~70 000 cells per cm^2); the volume of the medium was 75 μL; and 235 μL portions of the medium were added in the lower Transwell chambers. The medium was replaced every 2 to 3 days.

Estimation of the transport of chemotherapeutic agents. The transport of the drugs was estimated in a culture medium in which Gibco DMEM with a glucose content of 4.5 g L^{-1} was replaced by Gibco FluoroBrite DMEM without Phenol Red, while the other components remained the same. Dry weighed portions of cyclophosphamide and doxorubicin were dissolved in deionized water and 5-fluorouracil weighed portions were dissolved in DMSO; thus, base solutions were obtained. The working solutions were prepared by diluting base solutions with the Gibco FluoroBrite DMEM-based culture medium. The working concentrations were chosen as the highest concentrations of these drugs observed in patients′ blood during the treatment of breast cancer:[21] 5-fluorouracil, 25 mg L^{-1} (~0.192 mmol L^{-1}); cyclophosphamide monohydrate, calculated on a cyclophosphamide basis, 125 mg L^{-1} (~0.479 mmol L^{-1}); doxorubicin hydrochloride, calculated on a doxorubicin basis, 150 mg L^{-1}

(~0.276 mmol L^{-1}). In addition, a solution containing all of the three drugs in the same concentrations was prepared.

The drug transport was analyzed with complete replacement of the culture medium; 100 μL of the medium containing a single agent or the mixture of agents was poured into the upper Transwell chambers (see above) and 200 μL of pure culture medium was added to lower Transwell chambers. In the control experiment, the Gibco FluoroBrite DMEM medium without the drugs was used. Each drug was tested in 12 repetitions (12 Transwell inserts). Within 1 h after incubation with the chemotherapeutic agents, the medium from the upper and lower Transwell chambers was completely removed, the system was washed three times with a DPBS solution using 100 and 235 μL for the upper and lower chambers, respectively. Then the system was filled with the culture medium based on Gibco DMEM with 4.5 g L^{-1} glucose content.

HPLC MS analysis. Quantitative analysis of the test compounds in culture medium samples was performed with a LCMS 8030 HPLC MS setup (Shimadzu, Japan) consisting of a chromatograph and an electrospray ionization (ESI) triple-quadrupole mass spectrometer. The working solutions of chemotherapeutic agents in the culture medium and test samples of the culture medium were diluted with acetonitrile in 1 : 1 ratio, thoroughly mixed, and centrifuged for 5 min at 20 000 *g*. The supernatant liquid was transferred into vials and analyzed by HPLC MS (Phenomenex Luna C18 column, 4.6 mm×250 mm; 5 μm (Phenomenex, USA); flow rate of 1 mL min^{-1}; temperature of 40 °C; mobile phase *A*: 0.1 vol.% formic acid in water; mobile phase *B*, acetonitrile; gradient mode: 0—3.5 min, linear gradient from 2.5 to 100% phase *B*; 3.5—4 min, 100% phase *B*; 4—4.5 min, linear gradient from 100 to 2.5% phase *B*; 4.5—7 min, 2.5% phase *B*). The mobile phase was supplied to the ionization source under the following conditions: nebulizer gas flow of 3 L min^{-1}; drying gas flow of 10 L min^{-1}; desolvation line temperature of 300 °C; heating block temperature of 500 °C; negative ion mode for analysis of 5-fluorouracil; and positive ion mode for analysis of doxorubicin and cyclophosphamide. Quantitative analysis was carried out by multiple reaction monitoring (MRM mode) with parameters indicated in Table 1. The areas under the chromatographic peaks were calculated using the Skyline 4.1 software (MacCoss Lab, Department of Genome Sciences, University of Washington). Further data processing was done using the R 3.5 programming language with RStudio 1.1 integrated development environment.

Impedance spectrometry. The formation and the state of the cell monolayer was monitored using an impedance spectrometry instrument (BioClinicum, Russia) in the 20—20 000 Hz frequency range. The data of impedance spectrum measurements gave electrical characteristics of the monolayer such as transepithelial electrical resistance (TEER) and monolayer capacitance (*C*). The measurements were carried out on the 7th day after cell inoculation and then on the 8th day (before replacement of the culture medium by chemotherapeutic agents in the culture medium), after 1 h of incubation with the drugs, and on the 9th day (within 24 h after incubation with the drugs).

Table 1. MRM mode parameters for the MS analysis of the content of chemotherapeutic agents in the culture medium samples

Compound	*m/z*		Collision energy* /eV
	precursor ion	product ion	
Doxorubicin	544.15	397.05	13
Cyclophosphamide	261.00	140.00	23
5-Fluorouracil	129.05	42.00	16

* The collision energy of the precursor ions with the neutral gas in the collision cell; MS/MS in a triple quadrupole mass spectrometer.

Cell viability assessment. The cell viability was assessed using the CellTiter-Blue reagent kit (Promega) 24 h after cell incubation with the drugs (*i.e.*, on the 9th day after cell inoculation). This method is based on the ability of living cells to reduce the blue resazurin dye to pink fluorescent resorufin. After a certain period of time, the fluorescence signal of resorufin is proportional to the number of viable cells. In the cell viability experiments, the CellTiter-Blue reagent was mixed with the Gibco FluoroBrite DMEM-based medium in a ratio of 20 μL of the reagent per 100 μL of the medium. Then the medium was replaced by a medium with the reagent (120 μL in the upper chamber and 240 μL in the lower chamber). The cells were incubated for 1 h and then the reagent-containing medium in the upper and lower chambers was completely withdrawn and mixed. For fluorescence measurements, 150 μL of each mixture was added to each of two dark 96-well plates. Fluorescence was measured on a SpectraMax iD3 instrument (Molecular Devices, USA) at the excitation wavelength of 560 nm and emission wavelength of 590 nm. The cell viability was expressed in percent relative to the results of control experiments (without drugs).

Statistical data analysis. Statistical analysis for pairwise comparison of the results for two test groups was performed using the Student *t*-test. The values $p < 0.05$ were taken to be statistically significant. The numerical values in the text and in the plots are given as mean± standard deviation.

Results and Discussion

According to the impedance spectrum measured during the growth of BeWo b30 cells on permeable membranes of Transwell inserts, by the 7th day after inoculation at 70 000 cm^{-2} density, the cells form a confluent monolayer, since the impedance Nyquist plot for the cells has a typical semicircle shape. On this day, the TEER value reaches 40 Ω cm^2, while the monolayer capacitance is ~75 nF. The next day, before the incubation of cells with the anticancer drugs, TEER was ~90 Ω cm^2 and the monolayer capacitance was ~150 nF. In another study,[22] on the 6th day after inoculation of the BeWo b30 cells at 100 000 cells cm^{-2} density, the TEER was ~95 Ω cm^2, which is in line with our results.

The monolayer permeability of 5-fluorouracil, doxorubicin, and cyclophosphamide was analyzed by constructing calibrating plots in the concentration range of 150—0.0393 mg L^{-1} (altogether ten concentrations with a dilution step of 2.5). The drug concentrations were determined for the initial samples of the medium containing each drug or their mixture and for the samples of the medium above and below the cells 1 h after incubation of

the drugs with the cells. The calculated initial amount of the drug above the cells and the final amount of the drug above and below the cells were used to determine the drug amount that has passed through the placental barrier and the drug amount that remained bound to the cells (Table 2). Note that the model permeability of the placental barrier for all of the drugs did not show a statistically significant difference between the monotherapy and the FAC chemotherapy protocol.

The highest permeability was noted for cyclophosphamide, while the permeabilities of 5-fluorouracil and doxorubicin were markedly lower. According to DrugBank 5.0 data,[23] the partition coefficients log*P* for 5-fluorouracil, cyclophosphamide, and doxorubicin are –0.89, 0.8, and 1.27, respectively, that is, 5-fluorouracil is more hydrophilic, while cyclophosphamide and doxorubicin are more lipophilic. This accounts for better placental barrier permeability of cyclophosphamide than of 5-fluorouracil. It is also emphasized in one publication that, because of high hydrophilicity, the passive diffusion of 5-fluorouracil is complicated and the transport is saturable and is mainly performed by nucleoside transporter proteins.[24] However, doxorubicin penetrates the placental barrier model less efficiently than cyclophosphamide, although its log*P* is higher. Among other factors, this can be due to the higher molecular mass (543 g mol^{-1}) and binding to intracellular DNA and proteins for doxorubicin, which is indirectly confirmed by comparison of the initial and final amounts of doxorubicin in the culture medium after incubation with the cells: ~35% of the initial compound disappears. Previously,[25] it was shown that 78.3±7.6% of the introduced doxorubicin can be extracted from blood plasma and 58.4±7.3% can be extracted from solid tissues, which may be indicative of its active binding to proteins and DNA. Blood plasma proteins bind 50—85% of doxorubicin and only 12—14% of cyclophosphamide.[25] Furthermore, doxorubicin is a substrate for ABC transporter proteins, which transfer xenobiotics from cells to the external environment, thus preventing the drug accumulation and transport through the cells.[25]

A study of the drug transport through the placenta of pregnant mice demonstrated [26] that 1.5 h after administration of doxorubicin to the mother, 5.1±0.6% of the drug enters the fetal blood. In another study,[27] the concentration of doxorubicin in the mouse fetal plasma was found to be 5.0±0.2% of the concentration present in the mother blood flow. According to a study of anthracycline and cyclophosphamide transport through the placenta of pregnant baboons,[25] on average 7.5±3.2% of the doxorubicin administered to the mother enters the fetal blood circulation, while the concentration of cyclophosphamide in the fetal blood after drug administration to the mother is virtually identical to the concentration in the mother's blood when monitoring lasts for 2 h. An *ex vivo* perfusion of the human placenta showed[28] that ~2.96±0.75% of doxorubicin penetrates human placenta and that the degree of penetration does not depend significantly on the drug concentration. These results are consistent with our data on high placental barrier permeability of cyclophosphamide and low permeabilities of 5-fluorouracil and doxorubicin.

The impedance spectrum offers advantages over usual TEER determination, since not only TEER, but also the background resistance and electric capacitance of the cells can be derived from the impedance spectrum. Studies on intestine and placenta cell models demonstrated that the electrical capacitance can reflect the formation of microvilli on the cell surface during differentiation, while the background resistance can be associated with the formation of extracellular matrix by the cells.[6,29—32] In the analysis of changes in the electric characteristics of the cell model of placental barrier, it was ascertained that after incubation with the drugs for 1 h and replacement of the culture medium by a fresh portion, TEER decreases from ~90 to ~25 Ω cm^2; however, a similar change was also observed in a control experiment without chemotherapeutic agents.

Table 2. Penetration* of chemotherapeutic agents through the placental barrier model

Agent	Monotherapy				Mixture of three agents			
	Initial amount of the agent above the cells	Final of the agent		Amount of the agent remaining bound to the cells	Initial amount of the agent above the cells	Final amount of the agent		Amount of the agent remaining bound to the cells
		above the cells	below the cells			above the cells	below the cells	
5-Fluorouracil	2.788±0.198	2.518±0.062 (90.3±2.2)	0.041±0.007 (1.5±0.3)	0.230 (8.2)	3.145±0.210	2.439±0.147 (77.6±4.7)	0.041±0.008 (1.3±0.3)	0.665 (21.2)
Cyclophosphamide	13.564±0.065	11.624±0.061 (85.7±0.4)	0.992±0.093 (7.3±0.7)	0.949 (7.0)	12.231±0.060	11.168±0.468 (91.3±3.8)	0.975±0.028 (8.0±0.2)	0.088 (0.7)
Doxorubicin	13.441±0.181	8.704±0.361 (64.8±1.3)	0.148±0.161 (1.1±1.2)	4.589 (34.1)	14.314±0.197	9.221±0.546 (64.4±3.9)	0.043±0.021 (0.3±0.1)	5.050 (35.3)

* In μg; the value in parentheses is the fraction of the initial amount (%).

This suggests that the changes are attributable to the replacement of culture medium rather that to the action of the drugs on the cells (Fig. 1, *a*). Similarly, replacement of the medium results in decreasing capacitance of the cell layer from ~150 to ~120 nF (Fig. 1, *b*).

The TEER values of the cells within 24 h after cyclophosphamide treatment do not differ significantly from those of the control cells, but they are markedly lower than the TEER of the cells exposed to 5-fluorouracil (67.8±16.4 and 67.3±17.9 Ω cm^2 *versus* 90.0±20.1 Ω cm^2, respectively, see Fig. 1, *a*). Meanwhile, the capacitances of the cells after treatment with 5-fluorouracil and cyclophosphamide and untreated cells in the control experiment differ little, being 180±20, 168±18, and 161±21 nF, respectively (see Fig. 1, *b*). In the case of incubation with doxorubicin and with a mixture of the three drugs, the Nyquist plot was no longer a regular semicircle, which is indicative of nonconfluence of the monolayer. Therefore, the capacitance for these two cases decreases to zero. The TEER values for the cells 24 h after incubation with doxorubicin and a mixture of the three drugs were 16.2±3.3 and 16.1±1.9 Ω cm^2, respectively, which may also be indicative of mass cell death.

Analysis of the cell viability within 24 h after incubation with 5-fluorouracil and cyclophosphamide showed that it is close to that found in control experiments. No statistically significant difference is present between the cell viabilities found after incubation with doxorubicin or with three drugs, but they are considerably lower than the cell viability found in the control experiment, being 37±9 and 40±9%, respectively, relative to the control (Fig. 2).

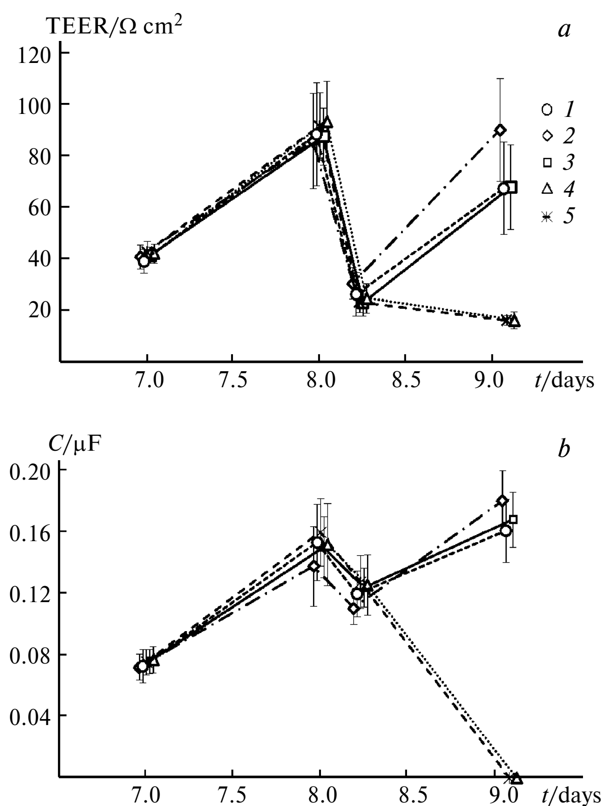


Fig. 1. Variation of TEER (*a*) and electrical capacitance (*b*) of the cell layer on exposure to anticancer drugs: control (*1*), 5-fluorouracil (*2*), cyclophosphamide (*3*), doxorubicin (*4*), a mixture of the three drugs (*5*).

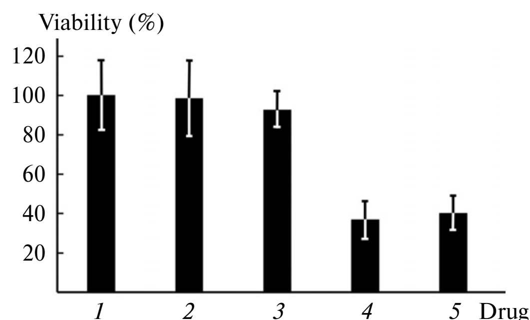


Fig. 2. Cell viability in the placental barrier model within 24 h after exposure to anticancer drugs: control (*1*), 5-fluorouracil (*2*), cyclophosphamide (*3*), doxorubicin (*4*), a mixture of the three drugs (*5*).

Thus, the proposed *in vitro* placental barrier model is suitable for the study of the transport and toxicity of organic compounds with different structures, molecular weights, and lipophilicities, which can be used for preclinical investigations of new compounds developed for the treatment of various human diseases in order to predict the placental permeability of the drugs and potential harm to the baby.

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References

1. F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, *CA. Cancer J. Clin.*, 2018, **68**, 394.
2. P. Brownbill, I. Chernyavsky, B. Bottalico, G. Desoye, S. Hansson, G. Kenna, L. E. Knudsen, U. R. Markert, N. Powles-Glover, H. Schneider, L. Leach, *Reprod. Toxicol.*, 2016, **64**, 191.
3. E. N. Knyazev, D. V. Mal´tseva, A. A. Zakharyants, G. S. Zakharova, O. V. Zhidkova, A. A. Poloznikov, *Bull. Exp. Biol. Med.*, 2018, **164**, 749.
4. E. N. Knyazev, D. V. Mal´tseva, A. A. Zacharyants, G. S. Zakharova, O. V. Zhidkova, A. A. Poloznikov, *Bull. Exp. Biol. Med.*, 2018, **164**, 757.
5. P. L. Grigsby, *Semin. Reprod. Med.*, 2016, **34**, 11.

6. S. V. Nikulin, E. N. Knyazev, T. N. Gerasimenko, S. A. Shilin, I. N. Gazizov, G. S. Zakharova, A. A. Poloznikov, D. A. Sakharov, *Mol. Biol.*, 2019, **53**, 411.
7. S. Conings, F. Amant, P. Annaert, K. Van Calsteren, *J. Pharmacol. Toxicol. Methods*, 2017, **88**, 25.
8. K. A. Fomicheva, A. I. Osip´yants, E. N. Knyazev, T. R. Samatov, M. Y. Shkurnikov, *Bull. Exp. Biol. Med.*, 2017, **162**, 792.
9. C. Blundell, Y.-S. Yi, L. Ma, E. R. Tess, M. J. Farrell, A. Georgescu, L. M. Aleksunes, D. Huh, *Adv. Healthc. Mater.*, 2018, **7**, 1700786.
10. J. S. Lee, R. Romero, Y. M. Han, H. C. Kim, C. J. Kim, J.-S. Hong, D. Huh, *J. Matern. Fetal. Neonatal Med.*, 2016, **29**, 1046.
11. E. N. Knyazev, G. S. Zakharova, L. A. Astakhova, I. M. Tsypina, A. G. Tonevitsky, G. T. Sukhikh, *Bull. Exp. Biol. Med.*, 2019, **166**, 321.
12. E. N. Knyazev, V. A. Petrov, I. N. Gazizov, T. N. Gerasimenko, I. M. Tsypina, A. G. Tonevitsky, G. T. Sukhikh, *Bull. Exp. Biol. Med.*, 2019, **166**, 369.
13. E. G. Rudimov, E. N. Knjazev, N. A. Khaustova, O. V. Grigorieva, L. B. Buravkova, *Dokl. Biochem. Biophys.*, 2017, **472**, 1.
14. C. N. Cordeiro, M. L. Gemignani, *Breast J.*, 2017, **23**, 200.
15. H. H. Yu, P. S. Cheung, R. C. Leung, T. N. Leung, W. H. Kwan, *Hong Kong Med. J. = Xianggang yi xue za zhi*, 2017, **23**, 387.
16. L. Knabben, M. D. Mueller, *Horm. Mol. Biol. Clin. Investig.*, 2017, **32**,
17. D. B. Longley, D. P. Harkin, P. G. Johnston, *Nat. Rev. Cancer*, 2003, **3**, 330.
18. A. Emadi, R. J. Jones, R. A. Brodsky, *Nat. Rev. Clin. Oncol.*, 2009, **6**, 638.
19. A.-M. Meredith, C. R. Dass, *J. Pharm. Pharmacol.*, 2016, **68**, 729.
20. C. J. Bode, H. Jin, E. Rytting, P. S. Silverstein, A. M. Young, K. L. Audus, *Methods Mol. Med.*, 2006, **122**, 225.
21. D. Jamieson, J. Lee, N. Cresti, R. Jackson, M. Griffin, J. Sludden, M. Verrill, A V Boddy, *Cancer Chemother. Pharmacol.*, 2014, **74**, 667.
22. H. Li, B. van Ravenzwaay, I. M. C. M. Rietjens, J. Louisse, *Arch. Toxicol.*, 2013, **87**, 1661.
23. D. S. Wishart, Y. D. Feunang, A. C. Guo, E. J. Lo, A. Marcu, J. R. Grant, T. Sajed, D. Johnson, C. Li, Z. Sayeeda, N. Assempour, I. Iynkkaran, Y. Liu, A. Maciejewski, N. Gale, A. Wilson, L. Chin, R. Cummings, D. Le, A. Pon, C. Knox, M. Wilson, *Nucleic Acids Res.*, 2018, **46**, D1074.
24. A. Takagi, T. Nishimura, T. Akashi, M. Tomi, E. Nakashima, *Drug Metab. Pharmacokinet.*, 2017, **32**, 151.
25. K. Van Calsteren, R. Verbesselt, J. Beijnen, R. Devlieger, L. De Catte, D. C. Chai, R. Van Bree, L. Heyns, J. de Hoon, F. Amant, *Gynecol. Oncol.*, 2010, **119**, 594.
26. K. Van Calsteren, R. Verbesselt, R. Van Bree, L. Heyns, E. de Bruijn, J. de Hoon, F. Amant, *Reprod. Sci.*, 2011, **18**, 57.
27. K. Van Calsteren, D. Hartmann, L. Van Aerschot, R. Verbesselt, R. Van Bree, R. D'Hooge, F. Amant, *Neurotoxicology*, 2009, **30**, 647.
28. P. Grohard, J. P. Akbaraly, M. C. Saux, S. Gimenez, J. Robert, A. Brachet-Liermain, J. J. Leng, *J. Gynecol. Obstet. Biol. Reprod. (Paris)*, 1989, **18**, 595.
29. S. V. Nikulin, E. N. Knyazev, A. A. Poloznikov, S. A. Shilin, I. N. Gazizov, G. S. Zakharova, T. N. Gerasimenko, *Mol. Biol.*, 2018, **52**, 577.
30. S. V. Nikulin, E. N. Knyazev, T. N. Gerasimenko, S. A. Shilin, I. N. Gazizov, G. S. Zakharova, A. A. Poloznikov, M. Y. Shkurnikov, *Bull. Exp. Biol. Med.*, 2018; doi:10.1007/s10517-018-4283-7.
31. D. Sakharov, D. Maltseva, E. Knyazev, S. Nikulin, A. Poloznikov, S. Shilin, A. Baranova, I. Tsypina, A. Tonevitsky, *BMC Syst. Biol.*, 2019, **13**, 19.
32. C. S. V. Nikulin, N. A. Mnafki Krainova, S. A. Shilin, I. N. Gazizov, D. V. Maltseva, *Mol. Biol.*, 2018, **52**, 583.

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