MULTIDRUG RESISTANCE OF TUMOR CELLS

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S u m m a r y. Multidrug resistance (MDR) developing during cancer chemotherapy is a clinically significant problem contributing to the failure of systemic cancer therapy, the result of which is the observable increase in the morality of patients. ABC transporters (ATP-binding cassette family) is one of the most numerous categories of proteins found in eukaryotic organisms, it contains the majority of multidrug resistant proteins. The proteins of the ABC family occur in various tissues (e.g. lungs, placenta, intestine, brain, heart, kidneys), and their main task is to transport drugs from the cell to the extracellular environment, which leads to a significant reduction in the intracellular concentration of the drug. According to current scientific knowledge, there are three generations of P-gp protein modulators. The first generation includes such pharmacological specimen as cyclosporin A and verapamil. The second generation – the specimens of this group are characterized by higher efficacy and lower toxicity compared to the I generation drugs, but they are not free from side effects. The third generation – specimens are characterized by high specificity towards P glycoprotein and do not affect the function of other transport proteins.

K e y w o r d s: multidrug resistance, tumor cells

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Multidrug resistance (MDR) developing during cancer chemotherapy is a clinically significant problem contributing to the failure of systemic cancer therapy, the result of which is the observable increase in the morality of patients. Resistance (MDR) means the cancer cells acquire the same non-sensitivity to the group of many cytostatics with a diverse structure and mechanisms of action [7].

During chemotherapy, cells exposed to one chemotherapeutic agent, initially sensitive, acquire cross-drug resistance to a large group of xenobiotics that differ in terms of function and structure. The MDR phenomenon is a cross-resistance to lipophilic substances, getting into the cell on the

basis of passive diffusion, being drugs with a different structure [14].

One of the main mechanisms of the emergence of drug resistance is the phenomenon over the expression of lamellar turners that causes too much drug to be removed from the inside of the cell into the extracellular space [5, 10]. Resistance (MDR) is connected with overlapping different phenomena and can be caused by complex mechanisms. You can count the most:

- high expression of genes encoding detoxification enzymes
- interruption of initiation and the course of process apoptosis (e.g. mutations inside the *TP53* gene anomalies of homeostasis of pro- and anti-apoptotic proteins).
- reduced uptake of the drug from the extracellular space
- change in the number of receptors and their affinity for cytostatic compounds
- changes in the rate of drug penetration into the cell
- inactivation or activation of pharmacological drug molecules in tumor cells
- irrigation of the cell cycle regulation, change of the duration of individual phases of the cycle
- mutation within the repair mechanisms of DNA, increase in the efficiency of the repair of genetic material
- removal of cytostatics into the extracellular space by the membrane transport proteins (mainly from the ABC protein group) [7].

In summary, it can divide resistance mechanisms into cytostatics into two large groups:

- mechanisms blocking the drug reaching the cell;
  - pharmacological indigestion, difficult to reach the drug to the tumor or impaired metabolism of the drug
  - disturbances in the transport of cytostatics into the cell; increased drug removal by the cell or reduced intake of the drug from the extracellular environment
- mechanisms blocking the action of cytostatics in target cell
  - changes in the target site for the drug, change in cell activity (increase or decrease), change in the function of target cells
  - increased repair of genetic material
  - changes in the functioning of cellular structures and in the regulation of apoptosis
  - metabolic changes, reduction of drug activation inside the cell, increase in drug catabolism [13].

Among all the above factors, the mechanism best known and appearing to have the greatest importance for the phenomenon of tumor resistance is the process of active cytostatic cell elimination through the membrane transported proteins, as a result of their abnormal, excessive expression [17]. Another factor that is of great importance for the process of cell's resistance to cytostatics is the participation of specific enzyme proteins. We include, first of all, transferase of glutathione and topoisomerase IIa [7, 11].

The human S-glutathione transferase belongs to the enzymatic family of glutathione transferases. It participates in the metabolic process of pharmacological compounds, participates in the protection of nucleic acids and lipids against peroxides, catalyzes the biotransformation of xenobiotics and prostaglandin isomerization. In the right cells is located in the cytoplasm. In precancerous and neoplastic lesions, an increased amount of this enzyme (exactly its isozyme pi) can be found in the cellar nucleus. The phenomenon applies especially to tumors resistant to such cytostatics as cisplatin, doxorubicin and alkylating compounds [4].

Topoisomerase IIa is an enzyme involved in DNA replication, necessary for the life of the cell. The cytostatic activity of such compounds as anthracyclines, mitoxantrone or etoposide is based on the formation of stable DNA connections and blocking the catalytic function of the enzyme topo IIa, leading to cellular apoptosis [7].

TRANSPORTED PROTEIN RELATED TO MULTIDRUG RESISTANCE

ABC transporters (ATP-binding cassette family) is one of the most numerous categories of proteins found in eukaryotic organisms, it contains the majority of multidrug resistant proteins [7].

The proteins of the ABC family occur in various tissues (e.g. lungs, placenta, intestine, brain, heart, kidneys), and their main task is to transport drugs from the cell to the extracellular environment, which leads to a significant reduction in the intracellular concentration of the drug [5]. These proteins accumulate in plasma membranes to be pumps-transporters responsible for penetration through lipid blends of relevant substrates (i.e. ions, hormones, lipids, drugs and other xenobiotics) despite the concentration gradient, to the external environment [6]. The transported proteins

also contribute to the maintenance of the cell's life, because it protects it from the toxic effects of exogenous substances, toxins and xenobiotics, which could have a negative effect on the functioning of the digestive system, liver or kidneys [5].

The special feature of transporters is their structure. ABC proteins are built to perform specific functions, which is why they have:

– TMD - transmembrane domain - presumably responsible for the connection of the substrate
– NBD - nucleotide binding domain - to which hydrolysis belongs.

The NBD domain contains the so-called Walker A and B motif, sequences directly involved in the ATP hydrolysis process, as well as the C motif (the so-called signature region) [5, 12].

Taking into account the functions possessed (e.g. transfer of various hydrophobic substances by external- and intracellular membranes), the ABC family proteins, found in many organisms, not only human, but also animal, vegetable or bacteria, play an important role in the processes of intracellular metabolism [3].

In eukaryotic cells, most ABC pumps contribute to the transfer of xenobiotics from the cytoplasm outside the cell or into intracellular compartments. These types of transporters include, among others P (P-gp) glycoprotein and MRP protein. Other proteins from the ABC family function as ion channels or regulators of these channels, e.g. cystic fibrosis protein - CFTR or EBCR [1, 2, 9].

In the cells of prokaryotic organisms, ABC proteins are primarily responsible for supplying from the external environment vital compounds that can not be obtained through diffusion, for example, carbohydrates, vitamins, metal ions [3, 5].

Transporters also take part in numerous metabolic processes, and disorders inside the genes coding for these proteins result in serious metabolic diseases; the deficiency of the multidrug-resistant protein MRP2 causes the Dubin-Johnson syndrome, the deficiency of MRP6 in turn is the cause of pseudo-xanthoma elasticum, a multifactorial mutation involving the skin, eyes and blood vessels [5].

ABC protein as a fundamental element of the bearing barrier and blood -the brain barrier also prevents exposure of sensitive nerve cells and cells of the developing fetus to harmful cytotoxic agents, regulating the permeability of the placenta cells and the central nervous system [15].

MULTIDRUG RESISTANCE MODULATORS

The unfavorable phenomenon of resistance of tumor cells to cytostatics is currently the main obstacle in achieving the success of cancer pharmacotherapy. Modern scientific and research works aim at the invention of methods reversing the processes of MDR.

The most extensive studies are currently being developed on modulators of multidrug P-glycoprotein resistance. Several methods of combating this resistance have been analyzed, including the use of cytostatics, on which cells with P-gp activity are affectionate, chemo – sensitive substances or antibodies with P-gp retarding effects high-dose chemotherapy and also the inhibition of MDR1 gene activity by suppressor substances [8, 18]. Studies carried out in the 1980s confirmed that blockers of the calcium channel (e.g. verapamil) can inhibit multi-drug resistance [16].

According to current scientific knowledge, there are three generations of P-gp protein modulators:

– The first generation includes such pharmacological specimen as cyclosporin A and verapamil. The use of these substances carries the risk of numerous side effects, in the case of verapamil it may be hypotensive as well as disturbances of the atrial ventricular conduction. The use of cyclosporin A in turn causes immunosuppression and is nephrotoxic. Modulators of the first generation rival with cytostatics for the place of binding of the drug on the P-gp molecule [10, 11, 12].
– The second generation – the specimens of this group are characterized by higher efficacy and lower toxicity compared to the I generation drugs, but they are not free from side effects. The preparation called Valspodar (PSC833), which is the representative of this group, is an analog of cyclosporine A but it is characterized by much greater blocking activity compared to the origin of it. It also does not have immunosuppressive properties.
– Connections such as Birikodar (VX-710), Dexguldipine or Dexverapamil are also more effective than I generative preparations, but they do increase the side effects of the chemotherapy. They also reduce the removal of xenobiotics by P-gp also from healthy cells causing an increase in the toxicity of anti-cancer therapy. It was also ob-

served that the compound being a modulator and cytostatic drug often competitively in relation to the activity of cytochrome P450 enzymes, which may have adverse effects on the metabolism of a healthy cell. The modulators of the II group also compete with cycostatics for the site of drug binding in the P-gp molecule [15].

– The third generation – specimens such as Tariquidar (XR9576), Laniquidar (R101933), Zosuquidar (LY3335979) are characterized by high specificity towards P glycoprotein and do not affect the function of other transport proteins. They do not exert their effects on cytochrome P450 and also do not significantly change the pharmacokinetics of the drug cells. Tariquidar, as the most promising compound of this group, binds to the P-gp molecule in a specific and non-competitive way with no other substances, the effect of this reaction is to block the transport functions of the P-gp. The affinity of XR9576 to P-gp is very large and the effect of the stronger reaction and more durable than in the case of I and II generation modulators[7].

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