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Review

Mechanisms of transport across cell membranes of complexes contained in antitumour drugs

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Abstract

Various mechanism of antitumour drug transport across cell membranes has been described. Particular attention has been paid to a passive transport, active transport and multidrug resistance of complexes contained in antitumour drugs. A drug supply to the target site depends on the blood circulation within the tumour, on characteristic drug diffusion in the tissue, and also on binding protein. The physiologic transfer of hydrophilic compounds across the membrane is usually intermediated by means of a specific receptor or a carrier in that membrane, which facilitates the transport of compounds to and from the cell. Some drugs, e.g. doxorubicin and annamycin, can pass across the membrane by intermediacy of liposomes which exhibit a great activity in penetrating into tumour cells. The efficiency of antitumour drugs is limited by the appearence of resistance, i.e. by the lack of sensitivity of the cell to the administered drug. The presence in the membrane of specific proteins belonging to the ABC carriers group is postulated in a resistance theory; they would be responsible for 'pumping out' lipophilic drug molecules from the cell. Participation of high-energy ATP molecule is required by *P*-glycoprotein (Pgp) and by MRP protein described in this paper for their action. The mechanisms that are responsible for the cell resistance to drugs have been presented by analysing the resistance to antimetabolites, particularly to folate and fluoropyrimidine analogues, to alkylating agents, e.g. cisplatinum, and to heterocyclic compounds being responsible for so-called multidrug resistance.

Keywords: Transport; Cell membranes; Antitumour drugs; Multidrug resistance

1. Introduction

Chemotherapy, which has been used for many years as one of the indispensable elements of cancer treatment is aimed at destroying tumour

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cells as well as simultaneously reducing damage to healthy cells of a patient. However, no compunds have been known hitherto which would selectively destroy the tumour cells. Namely, chemotherapeutic strategies that are used nowadays are mainly aimed at blocking the poliferation of the tumour cell by hampering metabolic processes which are necessary for the correct cell duplication. Procedures, which result in hampering such biochemical processes characteristic only for a particular tumour type, are also used, then it is the case of biochemical control of proliferation that takes place by means of drugs that are anologues of fundamental metabolites and co-factors, either natural or synthetic (Bishop, 1991).

A drug that is introduced into the organism undergoes a number of physical and chemical processes before it exerts its pharmacological effect. The essential condition of attaining a therapeutic effect of the drug is its penetration to the site of action with its suitable concentration. The drug transfer across the cell membrane is related to its penetration rate from blood to tissues as well as to its biological transformation and excretion.

Changes in electric charge distribution on and at a membrane surface, i.e. in a double electric layer, occur during the molecule or ion transport through biological membrane (Oksiejczuk & Figaszewski, 1997). The changes in composition and parameters of a double electric layer can be used to study the state of a cell membrane subjected to various factors, eg involved in the tumour transformation process.

Tumour cells produce and excrete to blood many substances that are present in the cell itself in trace amounts. These substances so-called biological tumour markers, have various physical and chemical properties and are classed into several groups: cancer-foetal antigenes, carcinogenic antigenes, proteins, cell metabolism products, hormones, enzymes, and isoenzymes. They are transported through the cell membrane to the blood circulation system.

Individual molecules that can traverse the membrane by various means, can be divided into two groups:

- the processes in which no direct energy supply is required, ie passive transport processes,
- the processes in which energy is directly consumed, i.e. active transport processes.

2. Passive transport

If uncharged molecules dissolved in water are suficiently small, they can move across a lipid bilayer down their concentration gradient directly by the way of simple diffusion. The examples are: ethanol, carbon dioxide and oxygen. However, most substances soluble in water can penetrate the membrane only in the presence of membrane transport proteins (carriers or channels) which are responsible for this transportation. The passive transport in the direction down the concentration gradient occurs spontaneously.

The carrier proteins bind specifically dissolved molecules and transport them across the lipid bilayer with changes in their conformation occurring in such a way that the site binding the carried molecule is open first on a side of the membrane and then on the other. Proteins of the channels form water-filled pores in the lipid bilayer and constitute the paths of diffusion. The transport by means of carriers can be either passive or active but the transport through channels is always passive. Most channel-forming proteins are selective ion channels allowing inorganic ions of determined charge and size to penetrate through the membrane down the electrochemical potential gradient. The transport through ion channels is at least thousand times faster than by means of any known carrier. Most ion channels are gated and then open but for a short period and only as a response to a specific stimulus, such as a change in membrane potential value or binding a ligand. Even when opened by a specific stimulus, the channels do not remain in this state for a long time; they randomly oscillate between the open and closed conformations (Kawiak et al., 1998; Alberts et al., 1999).

Numerous signalling substances, e.g. hormones, neurotransmitters, cytokinins and others, do not penetrate the cells but they are fixed by membrane receptor proteins hereby triggering a sequence of events resulting in a final biological effect. The reception of the signal starts in the site where the

signal produced outside the cell contacts a target molecule belonging to the cell. In principle, the target molecule is a receptor, i.e. a protein to be activated by one signal type only. A majority of receptor proteins of the cell surface belong to one of three main groups: ion channel receptors (ionotropic), receptors cooperating with G-type proteins (metabotropic) and enzyme-related receptors (catalytic) (Alberts et al., 1999; Konarska, 1995). If an outer-cell signalling molecule is bonded to a seven-helices membrane receptor then the latter undergoes a conformation change which deforms the cytoplasmatic side of the receptor, enabling it to interact with a G protein situated at the cytosol side of the cell membrane. G proteins are not uncommon in the nature, they belong to a large group of proteins that are able to bind guanosinetriphosphate (GTP) (Konarska, 1995). The G proteins differ from other proteins of this group by their trimeric structure. They are built of three subunits: α, β, and γ. The β and γ subunits form a stable complex, whereas the α subunit readily dissociates from the trimer. Other proteins able to bind GTP are monomeric and they are called small GTP binding proteins (Clapham & Neer, 1993). The G proteins are well suited to their function, i.e. to a rapid response to a stimulus, to triggering and shutting down a signal. Target proteins of the G protein subunits are either ion channels or membrane enzymes. The interaction of a G protein with an ion channel brings about an instantaneous change in the state and behaviour of the cell and interaction of those proteins provokes formation of further intracellular signalling molecules. The most frequent target enzymes of G proteins are adenylate cyclase which is responsible for formation of a small signalling molecule, cyclic adenosinemonophosphate (AMP), and also phospholipase C responsible for formation of small signalling units, inositol triphosphate and diacylglycerine (Alberts et al., 1999).

Some drugs that can overcome the defense mechanisms of the cell membrane can pass through the membrane by means of liposomes. Liposomes are closed spherical vesicles which are formed when a pure phospholipid is added to water. It is possible to enclose drugs in them. Liposomes are also used as highly efficient carriers. Compounds soluble in water can be enclosed in hydrophilic spaces of small liposomes, and lipophilic compounds can be included in their phospholipid layer. Drug distribution in a tissue can be markedly modified by the liposomes. Recent investigations of liposomes have revealed the existence of so-called 'stealth' liposomes containing hydrogenated phosphatidylinositol, ganglioside GM1 or other modified phospholipids (Gabizon et al., 1990). Furthermore, they show a marked activity in penetrating tumour cells.

3. Active transport

A carrier protein must execute some work to shift an ion or a molecule up against a concentration gradient. The concentration difference must be overcome by coupling the ion or molecule transfer with energy-supplying processes. The transfer across a membrane carried out in this way is called an active transport. It can be done only by specific carriers which are able to 'inject' specific energy sources to the process of transport. There are three main ways of active transport to and from the cell (Przestalski, 1989; Alberts et al., 1999; Konarska, 1995; Waksman et al., 1980):

- coupled carriers combine the transport of a molecule across the membrane up the gradient with the transport of another one occurring down the gradient,
- with an adenosinetriphosphate (ATP) driven pump, the transport up the gradient is coupled with the ATP hydrolysis,
- with light-driven pumps, occurring mainly in bacteria cells, the transport up the gradient is coupled with absorption of the energy of light.

The substance which should be transferred down the gradient must be earlier transported up the gradient using a form of active transport. Therefore the ATP-driven pumps of animal cell membrane remove the Na^+ ion from the cell up its electrochemical gradient and then the Na^+ ion flows back into the cell down its gradient. As Na^+ flows into the cytosol through the carriers coupled with Na^+, its flow is a driving force of active transfer of other numerous substances up their electrochemical gradient (Alberts et al., 1999).

The energy that is needed to transport the Na^+ ion out of the cell is obtained from ATP hydrolysis to ADP. In this way, it constitutes not merely a carrier but also an enzyme, transporting ATP-ase. Because the transport of Na^+ from the cell is coupled with the simultaneous transport of K^+ into it, the pump is also known as the Na^+ $^-$-K^+ ATP-ase pump or the Na^+-K^+ pump. It works incessantly removing Na^+, which is also incessantly reintroduced by other carriers and ion channels. In this way, the Na^+ concentration in cytosol is maintained 20–30 times lower and the K^+ concentration 10–30 times higher than those of surrounding liquid. In normal conditions, the electric potential of the cell cytosol is negative with respect to the environment in most cells and the ions have a tendency to penetrate the cells. In this way, the electrochemical driving force directed into the cell is great as it is the sum of the force caused by the concentration gradient and due to the electric potential gradient, both having the same direction.

The case of K^+ is different. The electric driving force is the same as that acting on the Na^+ ion, as it depends only on the ion charge, but the concentration gradient acts in the opposite direction. In normal conditions, the force driving the K^+ ion through the membrane is close to zero because the electric force driving the ion into the cell is almost completely compensated by the concentration gradient driving the ion out (Waksman et al., 1980):

The Na^+-K^+ pump operates in cycles. The Na^+ ion is bonded to the pump in the sites exposed to the cytosol (Stage 1) hereby initiating the ATP-ase activity. ATP is split and liberats ADP, and the phosphate group is bonded to the pump protein by a high-energy bond, ie the pump undergoes the process of phosphorylation (Stage 2). The phosphorylation stimulates the conformation change making it possible that the Na^+ ion be liberated from the outer surface of the cell and the K^+ binding site is exposed at the same surface (Stage 3). Binding K^+ from outside the cell triggers the process of releasing the phosphate group (dephosphorylation, Stages 4 and 5). The initial conformation of the pump is restored enabling it to release K^+ inside the cell (Stage 6). Thereafter, the whole cycle can be repeated (Mark et al., 1992). The energy is consumed only in the presence of the ions to be transported hereby avoiding useless ATP hydrolysis.

4. Multi-drug resistance

Multidrug resistance is the state, where the cells treated with a drug become resistant to this drug but also to other drugs, often structurally and functionally different. This action is characteristic particularly to natural heterocyclic compounds often used in therapy of tumours, e.g. the alkaloids (vinblastine, vincristine), antibiotics (actinomycin D, adriamycin, daunorubicin), podophylotoxin group compounds, cytochalazin D, and an alkylating agent — mitomycin. Their common characteristic feature is hydrophobicity (Kather & Ling, 1989; Mariani et al., 1989; Hindenburg et al., 1987; Broxterman et al., 1995; Kuzmich & Tew, 1991).

The tumour cells with multidrug resistance are characterized by lowered intracellular accumulation of the compounds they are resistant to. The low intracellular concentration of the administered drug in multidrug resistant cells compared with the unresistant ones, can be due either to less intense absorption by the cell or to intense elimination. Binding of the administered compounds within the cell must also be taken into account (Bashir et al., 1993). Probably, the drugs provoking multidrug resistance of the cells, penetrate the cells by the way of passive transport.

Accumulation of various drugs in acid medium in cytoplasmatic vesicles — lysosomes endosomes, Golgi apparatus vesicles — which are not 'target' sites of the drug and their removal from the cell during exocitosis must also be taken into account (Grzelakowska-Sztabert, 1995).

The multidrug resistance mechanism is related to the drug transport across the cell membrane. During the transport the cell receives not only necessary compounds for its correct functioning but also drugs; and harmful metabolism products are removed from it. A theory of drug resistance states that specific proteins belonging to the group of ABC transporters are present in the membranes (Twentyman, 1997); they are responsible

for 'pumping out' lipophilic drug molecules from the cell. The best known and best described are p-glycoprotein (Pgp) and an MRP protein accompanying multidrug resistance.

The most important property of p-glycoprotein is its ability to transport a diversity of hydrophobic compounds having either uncharged or positively charged molecules (Gottesman & Pastan, 1993; Chaudhary & Roninson, 1990). The mechanism of its action has not been fully explained; however, there are at least four theories of this phenomenon (Grzelakowska-Sztabert, 1995; Fardel et al., 1993; Endicot & Ling, 1989). It is sure that Pgp exhibits an ATP-ase activity (Zhang & Ling, 1995; Hamada & Tsuruo, 1988). It is stated in one of the theories that a channel is formed by *P*-glycoprotein in the cell membrane and that hydrophobic heterocyclic compounds are removed through it at the expense of energy originating from ATP hydrolysis (Fig. 1).

At the first stage, p-glycoprotein is phosphorylated by an energy-rich ATP molecule; which results in its conformation change. A site is uncovered in the peptide chain of great affinity to the compound to be transported. After the molecule is bonded, it is trasferred to the opposite membrane side, dephosphorylated; and it is followed by further conformation changes to liberate the transported molecule. The recognition pattern of the transported compound by Pgp is hitherto unknown. A hypothesis has been presented that the substrates that are removed from the cell by p-glycoprotein are 'marked' by intramolecular conjugates, eg with glutathione, under the influence of glutathione transferase (Ishikawa, 1990).

Another protein participating in the transport of xenobiotics out of the cell is MRP protein accompanying the multidrug resistance. It acts as the pump 'ejecting' the s-conjugates of glutathione as well as xenobiotic sulphates and glucuronates. ATP is required for the action of MRP like in the case of Pgp. (Leo et al., 1996; Jedlitschy et al., 1996).

5. Drug action mechanism

The prerequisite of the drug activity (Fig. 2) is its delivery to the target site.

It mainly depends on blood flow, in the cell on characteristic diffusion of the drug in the tissue and also on the kind of binding protein. Most

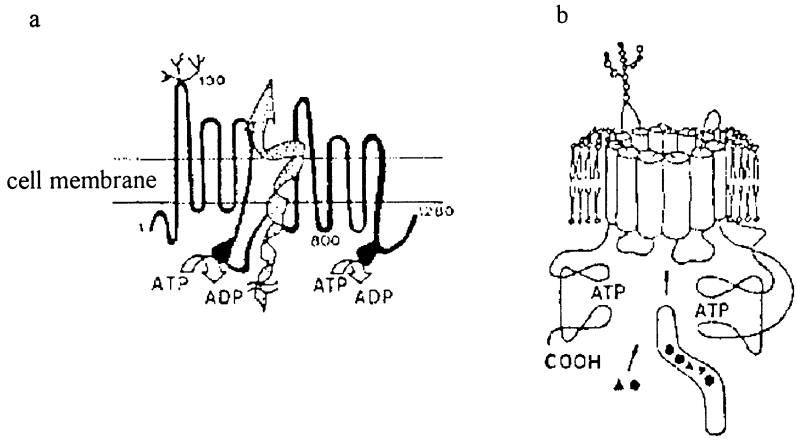

Fig. 1. Structure of p-glycoprotein. (a) Polypeptide chain of human p-glycoprotein presented as bold continuous line; the aminoacid positions are marked by digits. The ATP bonding sites at the cytoplasm side of the membrane are marked as well as fragments of carbohydrate chains (thin line) attached to the first polypeptide loop outside the cell membrane. The path of the removed compound through a hypothetic membrane channel is marked by the dotted arrow. (b) Model of p-glycoprotein as an energy-dependent pump forming a channel in the cell membrane. Twelve transmembrane domains are marked by cylinders along with a carbohydrate fragment attached to the peptide and two ATP binding sites. The compounds (filled polygons) which have penetrated into the cell can be removed through the channel opening either directly or as bonded to a hypothetic carrier protein.

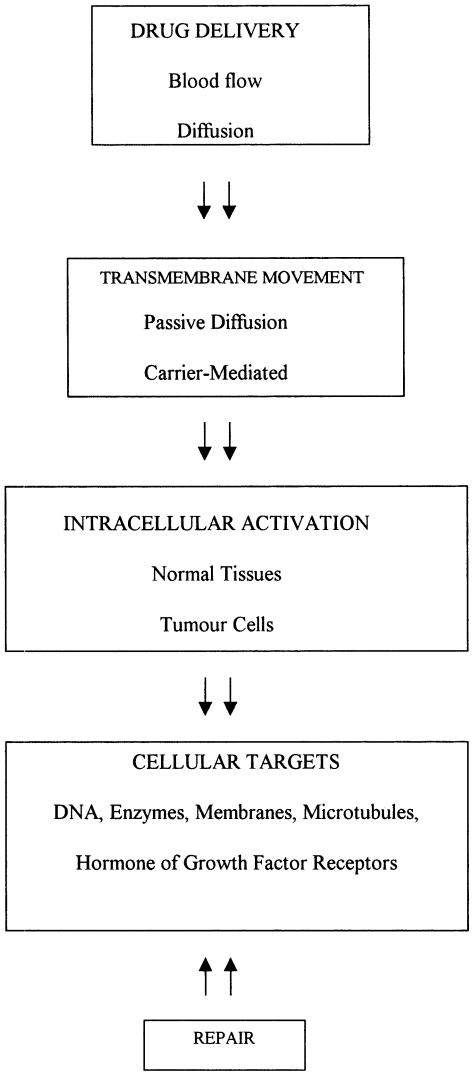

Fig. 2. Stages of drug action.

drug can become cytotoxic if they are accepted by the cell. Transfer of hydrophilic compounds of physiological importance across the membrane plasma is usually carried out by a specific receptor or carrier of the membrane, which facilitates the transfer of the molecule to or from the cell. The carrier-controlled transport systems are highly specific and they assure 'self-saturation' at high extracellular drug concentration. If the carrier-controlled transport system increases the drug flow into the cell, then not all carriers are able to bind the compounds against electrochemical forces (Mark et al., 1992). In this case, the intracellular drug concentration should be higher than the extracellular one. It can be attained under energy consumption which is usually provided by ATP.

Transmembrane movement is controlled by several factors, among them there are: the way of drug penetration into the tissue, the kind of transport and the essence of intracellular biochemical reactions causing the drug activity. The transmembrane transport should be limited by the drug activity (Mark et al., 1992). It is important to understand that the transmembrane transport often occurs in two directions and the final drug concentration in the cell represents the equilibrium between drug absorption and elimination.

The cells damaged by a cytostatic drug often exhibit various mechanisms of repair, and the cytotoxic effect of the drug is the result of damage and repair. They also strengthen mechanisms of repair related to cell resistance to those drugs. The relative rate of repairon DNA damages is relates to the sensitivity of the drug as well as its resistance (Wang et al., 1996). A detailed biochemistry of DNA repair of eucaryot is unknown. However, it is known that polymerase I of DNA contains 5′–3′ exonuclease which is active in repair of DNA damages. This activity enables polymerase I of DNA to perform 'translation cleavage' and instantaneous resynthesis of mutually completing DNA elements (Sekimizu, 1994).

Widely used antitumour drugs are (Grzelakowska-Sztabert, 1995):

- compounds inhibiting the synthesis of nucleic acid precursors — purines and pyrimidines,
- compounds interacting with DNA and the same inhibiting replication and translation processes,
- compounds inhibiting the synthesis of proteins or lipid cell membrane components,
- recently, the compounds preventing stimulation of cell divison by exogenic factors.

However, efficiency of such drugs is limited by appearing resistance, ie lack of cell sensitivity to the administered drug.

6. Platinum complexes

Cisplatin is the oldest and the most often used drug against testis and ovarian tumours, and recently also against head and bladder tumours. The oldest platinum complexes of antitumour activity are: cis-diaminedichloroplatinum(II) (Peyron salt) and cis-diaminetetrachloroplatinum(IV) (Fig. 3). This compound is most often administered in multidrug therapy combined with other antitumour drugs. Its cytotoxicity is mainly due to its interaction with DNA resulting in crosslinking DNA chains and in untypical DNA-protein bonds.

Membrane proteins are preferentially attacked by cisplatinum complexes, while the cisplatin bonds with phospholipids are relatively weak and reversible (Gea Speelmans et al., 1996; Todd & Hottendorf, 1995; Chu, 1994). However, the unstable interaction with phospholipids provokes changes in phospholipid conformation and in the structure and properties of the membranes, eg its permeability. The bond of platinum with monomeric protein also provokes conformation changes and perturbs self-association of the monomer (Wang et al., 1996). Binding of cisplatin to protein actine causes death of the cell by cross-linking, aggregation, depolymerizing and desorganisation of microfilaments.

The DNA-bonded proteins react with membrane phospholipids. Their activity in DNA replication, transcription and recombination is modified by acid phospholipids (Sekimizu, 1994).

A specific carrier active in cisplatinum transport into and out of the cell is still unknown. It seems that two membrane proteins: 48 kDa and 200 kDa participate in these processes; the latter is not identic with p-glycoprotein (170 kDa). Its role in removing cisplatinum from the cells has not been explained hitherto (Grzelakowska-Sztabert, 1995; Mark et al., 1992).

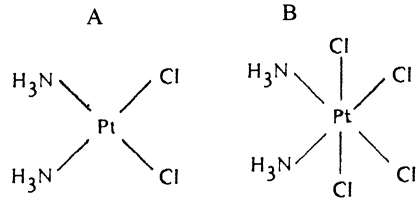

Fig. 3. Structure of complexes: A — diaminedichloroplatinum(II) and B — cis-diaminetetrachloroplatinum(IV).

Cisplatin does not penetrate into the cell directly under the action of Na^+, K^+-ATPase (Holler, 1993). It can be transferred across the cell membrane as hydrate or as bonded with small ligands like orthophosphates, nucleotids or amino acids.

Cisplatin affects the membrane activity by intermediacy of adenylate cyclase and by inhibition of AMP phosphodiesterase cycle. Phosphodiesterase enzyme includes cisplatinum into an isolated membrane fragment (Epand & Stafford, 1993). The process is started by adenylate cyclase activated by direct interaction with the metal complex or it occurs directly by binding cisplatin to the receptor of hormon, G protein or lipid molecule.

Cisplatin when administered as intravenous injections should be passively transported because of high chloride ion concentration in the blood plasma (about 0.1 M) but a part of the drug is hydrolyzed and deprotonated (Jedlitschy et al., 1996; Mark et al., 1992) (Fig. 2).

The fundamental component of the blood serum, albumine, has been found to react with platinum complexes binding them by the -SH groups of cysteine. A considerable part of the drug administered in this way is removed from the organism (up to 70% during first 24 h). The remaining part is transported by blood in unmodified form, it penetrates cell membranes in various organs, of the tumour cells too, as neutral complex by the way of passive transport. The retention time of the drug is different in various organs and it decreases in the sequence: kidneys > liver > lungs > sex organs > spleen > bladder > suprarenal glands > colon > heart > pancreas > small intestine > skin > stomach > brain (Wysokiński, 1998).

The chloride ion concentration in the cell drops to is about 4 mM, which provoks hydrolysis of the drug (Fig. 4). It has been demonstrated that in at least one chlorine atom of at least 42% molecules of the complex is replaced by a water molecule at such Cl^- ion concentration. It is

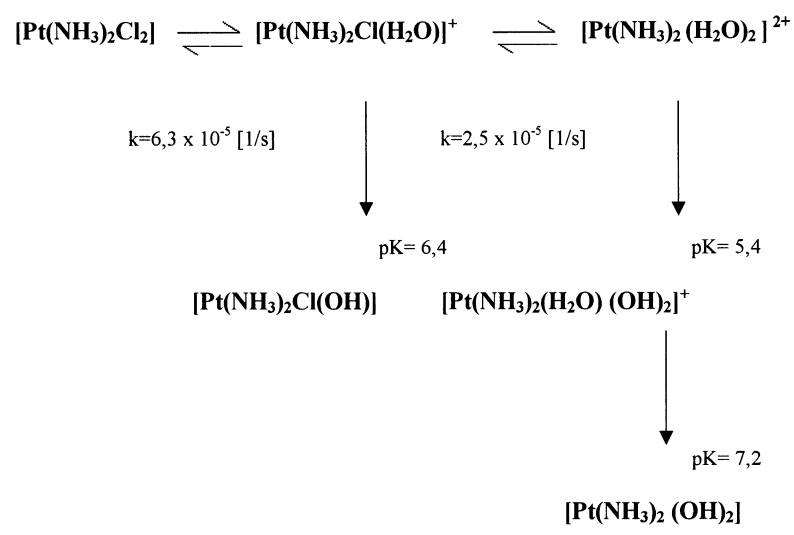

Fig. 4. Mechanism of cisplatinum hydrolysis inside the cell (Oldengurg et al., 1994).

supposed that just the cis-$[Pt(NH_3)_2(H_2O)Cl]^+$ and cis-$[Pt(NH_3)_2(H_2O)OH]^+$ ions show therapeutic effect (Wysokiński, 1998; Oldengurg et al., 1994). They are able to react with a variety of intracellular nucleophilic agents (DNA, RNA, proteins). They form bonds with methionine and with glutathione (Jedlitschy et al., 1994; Leier et al., 1994; Muller et al., 1994; Beck, 1987) and are transported in this form to their target-cell sites, i.e. to DNA. There, they form covalent bonds with the nitrogen atoms of guanine, adenine, and cytosine resulting in intra- and interhelical bonds with DNA. Cross-linking of deoxyrybonucleic acid perturbs the double helix structure and consequently inhibits DNA synthesis in tumour cells.

Various intracellular platinum contents have been observed during treatment of cells with platinum (II) complexes. The platinum content in the cell was very high in the leukemia P388/D1-type cells of mice (Holler, 1993). Initially, this accumulation has been explained by active transport of platinum(II) complexes initiating systems of transport during prolonged treatment and by restoring the levels characteristic with passive permeability of the membrane.

Because cisplatin exhibits a number of side effects (it damages kidneys, it provokes nausea and vomiting) and is inefficient in many types of tumour, the search of its analogues is continued in two directions. It is attempted to bind cisplatinum with biologically active compounds which should act as specific carriers (e.g. attempts to bind cis-Pt with estrogenes (Wysokiński, 1998) and new drugs are sought by introducing known antitumour drugs (e.g. vincristine, vinblastine, uracyl derivatives, histamine, aminosugars etc.) as ligands. The drugs are also looked for in which platinum should be replaced by other *d*-electron metals. Good therapeutical results have been obtained using some ruthenium(II), ruthenium(III), osmium(II), and palladium(II) complexes. Biological activity of some of these complexes proved to be higher than that of cisplatin. The ruthenium(III) complexes: [trans-$HIm(RuIm_2Cl_4)$], trans-imidazolium [tetrachlorobisimidazoleruthenate (III)] and [trans-$HInd(RuInd_2Cl_4)$], trans-indazolium [tetrachlorobisindazoleruthenate (III)] are of particular interest. They show much higher biological activity to some types of tumour in comparison with cisplatinum. Moreover, they are active to some cis-Pt unsusceptible ones. It results from the studies that the ruthenium(III) complexes are transported by albumines after they are introduced to the blood. They are coordinated by the imidazole nitrogen atom of histidine rests of albumine. Unfortunately, it can result in marked

changes in secondary and tertiary structure of protein and in blocking binding of heme to a marked degree (Trynda-Lemiesz & Kozlowski 1997).

7. Uracil derivatives

Fluoropyrimidines (Fig. 5), in particular 5-fluorouracil, have been used for over 30 years in therapy of tumours, mainly those of digestive tract (Mark et al., 1992; Grzelakowska-Sztabert, 1995).

These compounds are inactive in their original form and they can have a pharmacological effect only after they are subjected to a number of transformations caused by many enzymes after penetration into the cell (Berger et al., 1985) because they become effective compounds as mononucleotides and trinucleotides of fluoropyrimidines. Their action consists in inhibition of thymidylate synthase, the enzyme responsible for the de novo synthesis of thymine nucleotides. As the result, thymidynomonophosphate (dTMP) is not formed, the DNA synthesis is inhibited and this provokes death of the cell. In addition, fluoropyrimidine nucleotides can be incorporated into DNA and RNA instead of dTTP and UTP followed by various damages of nucleic acid molecules, eg by a cleavage of DNA helix. It has also been shown that uracyl derivatives can be bonded with platinum.

8. Folic acid derivatives

Folic acid derivatives (Fig. 6) are an important group of compounds exhibiting therapeutic action. They are used as antitumour, bactericide, and antiphlogistic drugs. Antifolates are not included into nucleic acids and, therefore, do not exhibit carcinogeneuos properties. Various folic acid derivatives: amino analogues (aminopterin, ametopterin), chinazoline derivatives (metasquine), pyrimidine analogues (trimetoprin, DDMP), triazine analogues and homofolates are counted among antifolates. Almost all folate analogues used hitherto are inhibitors of dihydrofolate reductase, a crucial enzyme in formation of tetrahydrofolate and coenzymatic forms of folate and, consequently, in biosynthesis of thymidylate and in DNA synthesis (Grzelakowska-Sztabert, 1983).

Resistance to antifolates can be the result of changes in properties and in amount of membrane proteins taking part in their transport into the cell. A membrane carrier participates in the transport of antifolates into the cell; in physiologic conditions it first of all transports hydrogenated coenzymatic folate derivatives. This transport is accompanied by the transport of anions across the membrane. A number of cells, particularly those adapted to grow in the presence of very low folate concentrations, also have a separate membrane carrier capable of transporting both hydrogenated and unhydrogenated folate derivatives

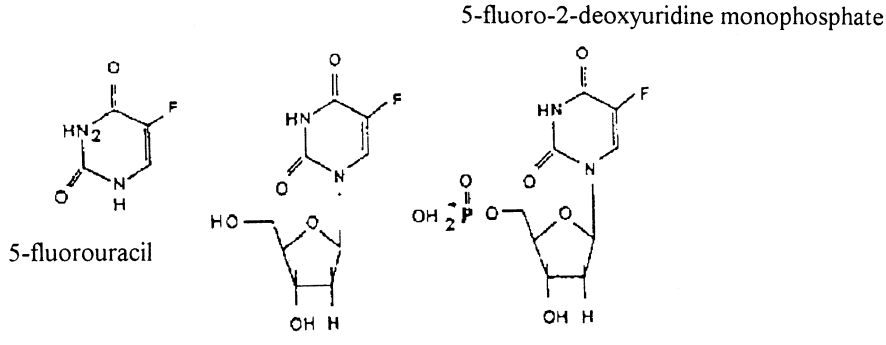

Fig. 5. Structure of fluoropyrimidines.

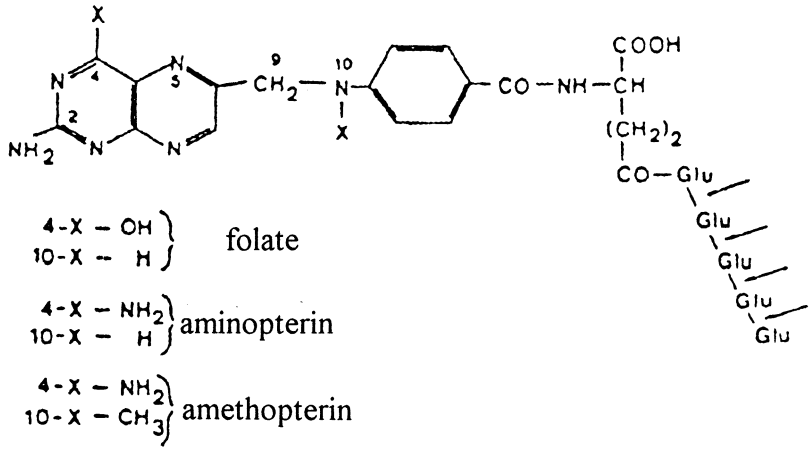

Fig. 6. Structure of folates and aminofolates-aminopterin and amethopterin. The bonds which can be split by γ-glutamylhydrolase are indicated with arrows.

(Henderson, 1986). Endocytosis of the transporting complex takes place in these cells. Resistant cells can be characterized by a lower affinity of membrane carriers to the antifolates which should be transported or by a reduced number of the carriers, probably as a result of a reduced expression of gene encoding the transport protein.

9. Heterocyclic compounds

Heterocyclic compounds (Fig. 7) have been used in therapy of tumours for many years. Their common characteristic is hydrophobicity caused by the presence of aromatic rings in their molecules and to positive charge at neutral pH. Owing to such a structure, the compounds penetrate into the cell by passive diffusion. The main site of action of anthracycline antibiotics is DNA. The antibiotics intercalate the nucleic acid bases inhibiting the DNA-dependent RNA synthesis and, at higher concentrations, the DNA synthesis, too. They can also be activated in the cell by microsomal enzymes. Radical forms of those compounds are then formed, they interact with DNA and they cause serious damage of chromosomes (K. Supino et al., 1988). It has also been shown that topoisomerase II, the enzyme controlling superhelical structure, can be the receptor of these drugs. Unfortunately, these compounds often provoke multidrug resistance making them inefficient in many instances. Probably, they are bonded by glutathione and are removed from the cell in such a 'labeled' form with contribution of p-glycoprotein.

It has been shown that the amino group at C-3 of the sugar rest plays a great role in the transport across cell membranes (W. Priebe, 1995). It constitutes the factor determining affinity of anthracyclines to the negative charge of phospholipids. The complex of doxorubicin with cardiolipine is strongly stabilized by interactions between the positively charged amino group and the negatively charged phosphate groups. The amino group is also an important factor stabilizing the anthracycline-DNA complex by electrostatic interaction of the positively charged amino group with the oxygen atom of the phosphate group of the nucleic acid. However, it also reacts with other macromolecules, eg with p-glycoprotein, provoking appearence of the multidrug resistance. Reduction of the amino group or replacing nitrogen by oxygen or sulfur increases its content and cytotoxicity in resistant cells.

It has been attempted to increase activity and effectivity of anthracycline antibiotics by closing them in liposomes which should facilitate their transfer through membranes. It was suggested in

earlier studies that it was possible to use liposomes to overcome multidrug resistance of the cells (Rahman et al., 1992). The liposomes should increase intramolecular accumulation of drugs which would otherwise be unable to pass through the cell membrane. Several clinic studies of doxorubicin-liposome preparations are being continued (Mayer et al., 1989). Hitherto, none of the tested preparations proved efficient against multidrug resistance. The liposomes seemed to be a good drug supply system but they were not tested in combination with the drugs characterized by great affinity to liposomes. The drugs were characterized by a high lipophility and limited solubility in water. Compatibility of anthracycline antibiotics with liposomes was studied. The liposome-drug preparation were obtained and were subjected to biological evaluation (Perez-Soler & Priebe, 1990). Anthracyclines were observed to be absorbed by liposomes in spite of their good solubility in water and then a transfer of compound out of the vesicle was observed. Annamycin proved suitable for preparation of a great variety of liposomes of different physical and pharmaceutical properties.

In recent years, electrochemical methods have been widely adopted to obtain information on DNA interactions with platinum group metals (Oliński & Zastawny, 1991). New biosensors with high and selective affinity these metals were studied. Behaviour of those molecules affected the mechanisms of the processes occurring at the electrode like those occurring at the membrane. The biosensors also modeled mutual interactions of nucleic acids in the membrane structure with nonadsorbed molecules by incorporating specific proteins and metals. The protein molecules can form complexes with platinum-group metals

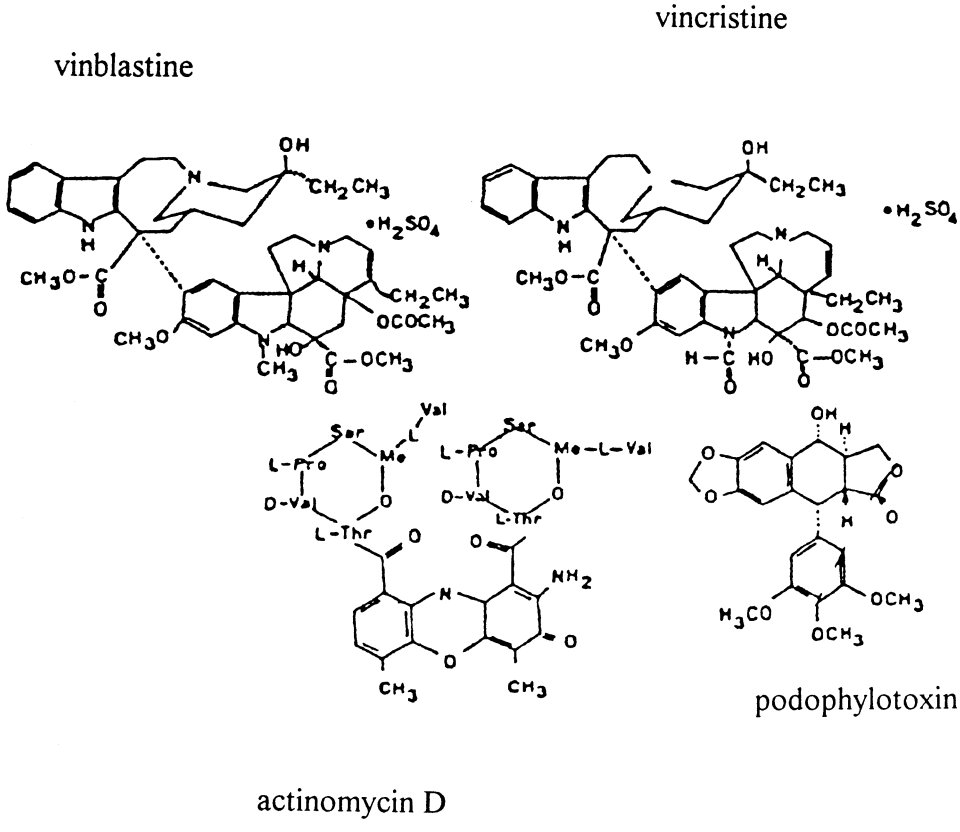

Fig. 7. Structure of some heterocyclic drugs which can provoke multidrug resistance.

which are able to control biological activity. A new biochemical sensor was elaborated based on a stationary mercury-oxygen electrode with silver substrate and a cellulose acetate membrane with included biomolecules. Such sensor is suitable to diagnose autoimmunological dieseases of humans and animals. It deals with the diseases causing anomally high DNA-specific antibodies in blood serum (Babkina et al., 1998).

Mass spectrometry and NMR spectrometry experiments have shown that interaction of cisplatin with phosphatidylserine (PS) results in the loss of two chloride ions and coordination of platinum to the amino and carboxy group of serine (Gea Speelmans et al., 1996). PS is exclusively located in the membrane plasma and belongs to about 20% phospholipids present there. Cisplatin forms specific complexes with phosphatidylserine not only in model membranes but also in the membrane plasma of human erythrocytes. Phosphatidylserine plays a decissive role in cell processes (Taylor et al., 1995; Igarashi et al., 1995) like signalling transduction, cell proliferation and apoptosis (death of cell). It is necessary for the activity of enzymes such as Na^+/K^+-ATPase and kinase C proteins. These functions can be markedly affected by interaction of cisplatinum with PS.

Doxorubicin, daunorubicin and other anthracycline antibiotics were studied to demonstrate similar electrostatic interactions with membranes. The differences in the general behaviour of drug-membrane system should be due to hydrophobic properties of the drug. Cyclic dichroism (CD) spectroscopy) elucidate conformation changes in the drug-membrane system (Gallois et al., 1998). Large monolayer vesicles (LUV) containing phosphatidylcholine, phosphatidic acid and cholesterol were used in this aim. Anthracycline-LUV interactions change molar proportions of phospholipids. At small changes in the drug-phosphatidylcholine system composition, the interactions depended on lipophilicity of anthracyclines. Thus, both doxorubicin and daunorubicin were bonded to the membrane as monomers and the signal of their cyclic dichroism in the visible range is positive. Doxorubicin having a low lipophilicity is bonded to LUV by electrostatic interaction with dihydroxyantraquinone molecule. Daunorubicin is unable to react electrostatically and hydrophobic interactions were detected in this case because it is more lipophilic while highly hydrophobic idaurubicin formed complexes of 2–3 idaurubicin molecules with one cholesterol molecule and with phosphatidic acid molecules inside the layer.

Kinetic activity of the drug was studied by the UV spectrophotometry, by pH-metric and potentiometric titration and by reversed-phase HPLC analysis (RP-HPLC). In the potentiometric technique, selective electrodes were used to measure concentrations of ions formed by hydrolysis of neutral drug precursors. A quantitative analysis of individual products was proposed using the HPLC method (Holler, 1993).

Long-lasting studies of transport mechanism of complexes contracted in the drugs has not solved many problems. New methods are sought to enhance the penetration of cytotoxic drugs across cell membranes and the known ones are further studied.

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