Oriental Journal of Chemistry Vol. **24**(2), 623-628 (2008)

Membrane-active agents -II

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(Received: January 20, 2008; Accepted: May 21, 2008)

ABSTRACT

Membrane-active agents may increase cell permeability to drugs, counteracting thereby drug resistance.

Key words: Membrane-active agents; Membrane-active peptides and protein.

INTRODUCTION

The *S. cerevisiae* zinc cluster transcription factors Pdr1 and Pdr3 mediate general drug resistance to many cytotoxic substances also known as pleiotropic drug resistance (PDR). Exposure of yeast cells to 2,4-dichlorophenol (DCP), benzyl alcohol, nonionic detergents, and lysophospholipids causes rapid induction of Pdr1 and Pdr3. Furthermore, Pdr1/Pdr3 target genes encoding the ABC proteins Pdr5 and Pdr15 confer resistance against these compounds. Genome-wide transcript analysis of wild type and *pdr1*Δ *pdr*3 Δ cells treated with DCP reveals most prominently the activation of the PDR response but also other stress response pathways. Polyoxyethylenelaurylether treatment produced a similar profile with regard to activation of Pdr1 and Pdr3 suggesting activation of these by detergents. The Pdr1/Pdr3 response element (PDRE) is sufficient to confer regulation to a reporter gene by these substances in a Pdr1/Pdr3-dependent manner. Compounds with potential membrane-damaging or perturbing effects might function as an activating signal for Pdr1 and Pdr3 and suggest a role for their target genes in membrane lipid organization or remodelling[15].

A class of polymaleic anhydride polymers capable of disrupting cell membranes. Co-delivery of these polymers with biologically active compounds increases cellular cytoplasmic delivery of the compounds.

The mode of action of cervinomycin, which is a new antibiotic active against Grampositive bacteria including anaerobes, was studied in *Staphylococcus aureus* using triacetylcervinomycin A_1 (ACVM), an acetyl derivative of cervinomycin A_1. ACVM stimulated the leakage of UV_{260}-absorbing materials, amino acids and potassium ions from resting cells and protoplasts. Phospholipids partially reversed the inhibitory activity of ACVM in a growing culture. ACVM interact with phospholipids in the cytoplasmic membrane and then interfere with the membrane transport system[16].

Carcinogen aflatoxin B_1 (AFB_1) was found to be a potent clastogen for phytohemagglutinin stimulated human lymphocytes. It also induced sister chromatid exchanges. These types of chromosomal damage were induced at very low levels of covalent AFB_1-DNA adducts suggesting that AFB_1 operates in part by indirect action because

of its membrane-active character. The membrane-active character of AFB_1 is due to: (i) AFB_1 stimulated the excretion of hydroxy- and/or hydroperoxy-arachidonic acid (AA) and free AA into the culture medium; (ii) the phospholipase A_2 inhibitor *p*-bromophenacylbromide was anticlastogenic; (iii) the inhibitors of the oxidative metabolism of AA indomethacin, flufenamic acid, 5,8,11,14-eicosatetraynoic acid, nordihydroguaiaretic acid and BN 1015 were anticlastogenic[17].

The membrane-active polyether ionophores are lipid-soluble molecules that transport polar cations across the cell membranes. Lasalocid, a polyether carboxylic acid ionophore, was isolated from *Streptomyces lasaliensis*. It disrupts membrane potential and stimulates ATPase activity in mitochondria. Nigericin, isolated from *Streptomyces hygroscopicus*, is another polyether ionophore which exerts similar activity .Today only lasalocid is therapeutically useful: it is an effective anticoccidial drug for poultry and farm animals. The vertebrate polycationic peptides demonstrate a broad spectrum of antimicrobial activity. Their mechanisms of action are: perturbation of the membrane function, formation of transient channels, and attachment to cytosolic targets are those recently proposed[18].

Chemically diverse compounds having potent in vitro anti-HIV activity were found to block ionophore-induced Ca(2+) influxes. The compounds included polyanionic species such as dextran sulfate and suramin, as well as prostatin, oligophosphorothioates and oxathiin carboxanilide. The blocking activities were dose-dependent and occurred within the same concentration ranges as their respective antiviral activities[19].

Leishmanicidal drugs interacting stoichiometrically with parasite plasma membrane lipids, thus promoting permeability, have raised significant expectations for Leishmania chemotherapy due to their nil or very low induction of resistance. Inherent in this process is a decrease in intracellular ATP, either wasted by ionic pumps to restore membrane potential or directly leaked through larger membrane lesions caused by the drug. Adapted a luminescence method for fast automated real-time monitoring of this process, using Leishmania donovani promastigotes transfected with a cytoplasmic luciferase form, previously tested for anti-mitochondrial drugs. The system was first assayed against a set of well-known membrane-active drugs [amphotericin B, nystatin, cecropin A-melittin peptide CA(1-8)M(1-18)], plus two ionophoric polyethers (narasin and salinomycin) on Leishmania, then used to screen seven new cecropin A-melittin hybrid peptides. All membrane-active compounds showed a good correlation between inhibition of luminescence and leishmanicidal activity. Induction of membrane permeability occurs by dissipation of membrane potential[20].

A 30-min treatment of MCF-7 cells with 1 Âµmol of valinomycin per liter resulted in absence of red fluorescence from JC-1, indicative of dissipation of mitochondrial membrane potential[18]. F-FDG incorporation was significantly increased by 30 min of treatment with valinomycin and was still apparent after 3.5 h of incubation. Hexokinase activity and subcellular distribution were not significantly different between control cells and cells treated for 30 min with valinomycin. Glucose transport was moderately though significantly increased, and lactate production was also increased[21]. To develop new approaches for the treatment of invasive infections caused by *Aspergillus fumigatus*, the in vitro interactions between itraconazole (ITZ) and seven different nonantimicrobial membrane-active compounds amiodarone (AMD), amiloride, lidocaine, lansoprazole (LAN), nifedipine (NIF), verapamil, and fluphenazine against seven ITZ-susceptible and seven ITZ-resistant (ITZ-R) strains were evaluated by the checkerboard microdilution method based on National Committee for Clinical Laboratory Standards M-38A guidelines.Statistically significant synergy was found for the combination of ITZ and AMD and the combination of LAN and NIF, although with different intensities against ITZ-R strains. The FIC index values ranged from 1 to 0.02 for ITZ-AMD, 0.53 to 0.04 for ITZ-LAN, and 0.28 to 0.06 for ITZ-NIF. By use of the BI-based model, the strongest synergy was found for the combination of ITZ with AMD, followed by the combination of ITZ and NIF. The combination of ITZ with calcium pump blockers displayed in vitro synergistic activity, primarily against ITZ-R strains[22].

The in vitro activities of buforin II, cecropin P1, and magainin II, alone and in combination with six clinically used antimicrobial agents, against 12 clinical isolates of *Stenotrophomonas maltophilia* were investigated. Antimicrobial activities were measured by MIC and time-kill studies. The isolates were susceptible to the peptides at concentrations in the range of 0.50 to 16 Âµg/ml. Synergy was observed when the peptides were combined with polymyxin E, meropenem, ceftazidime, piperacillin, and clarithromycin[23].

Procaine, a membrane-active anesthetic, potentiates uptake of puromycin and dexamethasone in the variantsMS23 of the murine thymoma cell line W7[24].

Gossypol was bound to human erythrocytes and cell membranes isolated from erythrocytes. 4,4'- Diisothiocyanostilbene-2,2'-disulfonic acid is a potent inhibitor of anion transport and can be covalently bound to band 3. Covalently bound 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid blocked a fraction of gossypol binding to erythrocyte membranes[25].

Treatment of CHO cells with drugs which are known to increase membrane lipid fluidity reduced the cells' ability to adhere to protein coated substrates. The concentrations of local anesthetics, nonionic detergents or aliphatic alcohols required to reduce CHO cell adhesion by 50% were similar to those reported to block nerve conduction, indicating that these drugs can affect the membrane at physiologically significant concentrations. Nonionic detergents and aliphatic alcohols, but not local anesthetics, caused increases in the fluidity of CHO plasma membranes (measured by fluorescence polarization) at concentrations which inhibited cell adhesion[26].

Ether-linked glycerophospholipids (ether lipids, EL) are selectively toxic and anti-proliferative agents against cancer cells *in vitro*.Their mechanism of action is mediated through an interaction with the plasma membrane and the membrane lipid composition may modulate it. Cholesterol concentration modulates EL toxicity in the K562, U937 and MOLT4 leukemic cell lines *in vitro*. Cells become sensitive to otherwise ineffective doses of EL when their cholesterol content is lowered. Cell cholesterol levels were reduced by exposure to an egg lipid mixture (neutral glycerides, phosphatidylcholine and phosphatidylethanolamine, AL721). This helps in understanding of the EL mechanism of action on membranes and the cellular cholesterol concentration must be considered a major factor in modulating the cytotoxic effects of EL[27].

In addition to primaquine, a group of membrane-active drugs, specifically hydrocortisone, vinblastine, and chlorpromazine can induce membrane internalization in erythrocytes. This is a metabolic process dependent on drug concentration, temperature, and pH[28].

Membrane-active agents reduce endotoxin toxicity in vivo decreased endotoxin binding to erythrocyte membranes in vitro, with propranolol and pranolium being the most effective.The in vivo accumulation of 51Cr-endotoxin in guinea-pig lung was reduced by prior treatment with (+)-propranolol or pranolium, paralleling the results of the in vitro binding studies. Membrane-active agents such as (+)-propranolol may be useful adjuncts to antimicrobial drugs in the therapy of gram-negative endotoxaemia[29].

The lipid composition of Prototheca wickerhamii ATCC 16529 has unique susceptibility of the organism to drugs of three membrane-active antimicrobial classes: the polyenes, the polymyxins, and the imidazoles. The presence of ergosterol in the neutral lipid fraction of the membrane is likely responsible for the exquisite susceptibility to amphotericin B. The presence of a large quantity of free fatty acids in the membrane appears responsible for imidazole susceptibility. The membrane determinants of polymyxin B susceptibility are less well defined[30].

Verapamil is used clinically as a Ca^{2+} channel inhibitor for the treatment of various disorders such as angina, hypertension and cardiac arrhythmia. The effect of verapamil on the bacterium Escherichia coli show inhibition cell division at growth sub inhibitory concentrations, independently of the SOS response. Verapamil is a membrane active drug, with similar effects to dibucaine, a local anesthetic. Thus, both verapamil and dibucaine

abolish the proton motive force and decrease the intracellular ATP concentration. This is accompanied by induction of degP expression, as a result ofthe activation ofthe RpoE (SigmaE) extra-cytoplasmic stress response, and activation ofthep.sp operon. Such effects of verapamil, as a membrane active compound, could explain its general toxicity in eukaryotic cells[31].

The amphiphilic membrane-active cationic drugs dibucaine and propranolol block BH3 peptide-initiated cyt c efflux by preventing the integration of Bax into the mitochondrial outer membrane. BH3 peptide-initiated release of cyt c from GT 1-7 neural cell mitochondria was inhibited by dibucaine and propranolol at concentrations of 100-300 ìM. Recombinant Bax (100 nM) alone did not release cyt c from adult rat brain mitochondria; however, when BH3 peptide or caspase-8 cleaved Bid (cBid) was added, robust cyt c release was achieved that was inhibited completely by 200 µM dibucaine or propranolol. Dibucaine and propranolol inhibit Bax-induced permeability changes through a direct interaction with the lipid membrane and present a novel target for the development of neuroprotective, antiapoptotic therapeutics[32].

Hygromycin B, an inhibitor of protein synthesis in eukaryotic cell-free systems, does not block translation in intact HeLa cells, as mammalian cells are normally impermeable to this antibiotic. However, the presence in the culture medium of the ionophore nigericin at concentrations that did not interphere with protein synthesis but rendered human HeLa cells permeable to hygromycin B.A similar effect was achieved with a number of ionophores, such as valinomycin, monensin, gramicidin D, bromolasalocid, A23187, amphotericin B and nystatin, as well as with the cardioglycoside antibiotic ouabain, which blocks (Na^+, K^+)-ATPase activity. The inhibition of protein synthesis by destomycin A, anthelmycin, gougerotin, edeine, tetracycline and several nucleotide derivatives in cultured HeLa cells was greatly enhanced by the presence of 0.8 Î¼M nigericin. Concentrations of Î±-amanitin above 200 Î¼/ml were required to inhibit RNA synthesis completely in intact cells and the presence of nigericin reduced 20-50-fold the concentration of Î±-amanitin necessary to inhibit transcription[33].

The anti-viral activity of D4T and AZT is limited by their ability to form the 5' triphosphate derivatives which are believed to act as chain terminators of reverse transcription. Studies in the SIVmac model could be used to develop strategies to increase the anti-viral effects of these compounds. The in vitro activity of AZT and D4T against SIVmac can be increased by combination with a membrane active compound DPM[34].

Peptides derived from viral sequences such as the N-terminus of influenza virus haemagglutinin HA-2, the N-terminus of rhinovirus HRV2 VP-1 protein, and other synthetic or natural sequences such as the amphipathic peptides GALA, KALA, EGLA, JTS1, or gramicidin S have been tested. Ligand–polylysine-mediated gene transfer can be improved up to more than 1000-fold by membrane-active compounds. Other polycations like dendrimers or polyethylenimines as well as several cationic lipids provide a high transfection efficiency. Electroneutral cationic lipid–DNA complexes however can be strongly improved by the addition of membrane-active peptides[35].

The various amide surrogates, novel pseudopeptides like the peptide were active against current drug resistant fungi and pathogenic fungi isolated from patients, and also had a strong synergism with current antifungal drugs against *Candida albicans*. The leakage assay suggested that the pseudopeptides also acted on the lipid membrane of pathogenic cells. The novel pseudopeptides had advantages over the peptide as a candidate for a novel antifungal drug and backbone modifications can be a tool in the development of a novel antifungal agent from membrane-active peptides isolated from natural sources or chemically synthesized[36].

Spectrin specifically binds to CPZ intercalated into inside-out vesicles depleted of all peripheral proteins. CPZ-induced mechanical stabilization of the erythrocyte ghost membranes may be mediated by direct binding of spectrin to the bilayer. Membrane active drugs that partition into lipid bilayer can thus induce cytoskeletal protein interactions with the membrane and modulate membrane material properties.[37]

The natural triterpene betulinic acid and its analogues (betulinic aldehyde, lupeol, betulin, methyl betulinate and betulinic acid amide) caused concentration-dependent alterations of erythrocyte membrane shape towards stomatocytes or echinocytes according to their hydrogen bonding properties. Thus, the analogues with a functional group having a capacity of donating a hydrogen bond (COOH, CH_2OH, $CONH_2$) caused formation of echinocytes, whereas those lacking this ability (CH_3, CHO, $COOCH_3$) induced formation of stomatocytes[38].

Inhibition of phospholipase C and/or PKC is currently thought to be the main biochemical target of alkylphosphocholines. Considering the great importance of both phosphoinositide metabolism and the regulation of a broad spectrum of cellular functions, including differentiation and invasion, miltefosine could become an interesting new drug[39].

The thermophilic eubacterium *Bacillus stearothermophilus* has been used as a model system to identify DDT-promoted events in biological membranes putatively related with the insecticide toxicity.Growth inhibition by DDT is concentration-dependent, being attenuated or removed by the addition of 2.5-mM Ca^{2+} to bacterial cultures. Growth and viability of bacterial cells are affected by DDT concentrations lower than those able to induce detectable bulk fluidity alterations, indicating high sensitivity of the intact bacterial system to alterations in limited membrane domains not directly probed by fluorescent probes[40].

The multidrug-resistance (MDR)-reversing ability of the catamphiphilic drugs could be mediated through their interaction with the membrane phospholipids directly (through changes in membrane permeability and fluidity) and/or indirectly (through inhibition of P-glycoprotein phosphorylation via inhibition of the phosphatidylserine-dependent protein kinase C or changes in the conformation and functioning of the membrane-integrated proteins via changes in the structure organization of the surrounding membrane bilayer) to the reversal of MDR. *Trans*- and *cis*-flupentixol were found to interact most strongly with both the phospholipids, followed by trifluoperazine, chlorpromazine, triflupromazine, flunarizine, imipramine, quinacrine and lidocaine. Differences in the interaction of *trans*- and *cis*-flupentixol with the phospholipids are responsible for their different MDR-reversing ability. Verapamil showed moderate membrane activity, assuming that the membrane interactions are not the only reason for its high MDR-reversing ability. Amiodarone showed very strong interactions with phosphatidylserines and is recommended for further MDR-reversal studies[41].

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