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Membrane-active agents -I

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ABSTRACT

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

Key words: Membrane-active agents; membrane-active peptides and proteins.

All organisms need protection against microorganisms, e. g. bacteria, viruses and fungi. For many years, attention has been focused on adaptive immunity as the main antimicrobial defense system. However, the adaptive immune system, with its network of humoral and cellular responses is only found in higher animals, while innate immunity is encountered in all living creatures. The turning point in the appreciation of the innate immunity was the discovery of antimicrobial peptides in the early eighties. In general these peptides act by disrupting the structural integrity of the microbial membranes. Membraneactive peptides and proteins play a crucial role in both the innate and the adaptive immune system as antimicrobial agents[1] Naturally occurring peptides and protein domains with amphipathic sequences play a dominant role in physiological, lipid membrane-reorganizing processes like fusion, disruption, or pore formation. More recently this capacity to modulate membrane integrity has been exploited for drug delivery into cells. Incorporation of synthetic membrane-active peptides into delivery systems has been found to enhance intracellular delivery of drugs including oligonucleotides, peptides, or plasmid DNA. In the majority of applications, the amphipathic peptides are designed to act after uptake by endocytosis, releasing the delivered agent from intracellular vesicles to the cytoplasm. Alternatively, peptides might mediate direct drug transfer across the plasma membrane. Although encouraging results have been obtained with the use of synthetic peptides to enhance cellular delivery of various compounds, the naturally evolved mechanisms observed in the entry of viruses or protein toxins are still far more efficient. For the development of improved synthetic peptides and carrier systems a better understanding of the molecular details of membrane-destabilization and reorganization will be essential[2]. Membrane active drugs: local anaesthetics, tri-cyclic antidepressants, beta blockers, antihistamines.

Use of liposomal drug delivery systems can enhance the therapeutic potential of membrane active anti-cancer and anti-infectious drugs. Thus, the therapeutic index of the important antifungal agent amphotericin B is markedly improved via incorporation of the drug into liposomes. The mechanistic basis of this effect seems to be an increase in the selectivity of the drug at the cellular level. Thus, free amphotericin B can readily partition into both fungal and mammalian membranes and can cause toxicity to both types of cells, giving rise

to the notorious in vivo toxicity of this drug. By contrast, when amphotericin B is formulated in certain types of liposomes, the drug still readily partitions into fungal membranes but can no longer partition into animal cell membranes, thus markedly reducing its toxicity. Liposomes can also be used to reduce the toxicity of membrane-active antitumor drugs. Thus, the peptide ionophore valinomycin is far less toxic to animals when presented in liposomal form. Nonetheless, the drug retains useful antitumor activity in this form. The underlying basis of the enhanced therapeutic index of liposomal valinomycin is unknown at this time but is being explored. The development of membrane-active anti-tumor drugs, in conjunction with liposomal delivery systems, could be an important new approach in cancer chemotherapy. While no anticancer drug is likely to be free of toxic side effects, the toxicities engendered by membrane-active antitumor drugs are likely to affect a different spectrum of tissues and organs than those caused by "conventional" antitumor drugs. Thus membrane-active drugs could complement existing drugs and provide a valuable adjunct to therapy[3].

The treatment of metastatic growth still constitutes a challenge for cancer research. Tumor progression is often accompanied by a loss in sensitivity to previously efficient drugs. Decreased intracellular accumulation of cytotoxic agent is probably the major reason for drug resistance, although other mechanisms have also been described. Properties of the cell membrane have been shown to determine the metastatic phenotype. This cellular organelle is also responsible for the multiple drug resistance phenomenon. Membrane-active agents may increase cell permeability to drugs, counteracting thereby drug resistance[4].

The cytotoxicity of glucosamine was potentiated by the local anesthetic lidocaine, and by other membrane-active drugs, at concentrations that were growth inhibitory but nonlytic.The inhibition of the incorporation of [2-14Clacetate into sterols was also potentiated by lidocaine.Other membrane-active drugs also potentiated the cytotoxicity of glucosamine :an aromatic amine(adiphenine), a benzylisoquinoline (papaverine), a butyrophenone (haloperidol), a cyclohexanone (ketamine), a phenothiazine(thioridazine), and a thioxanthine (chlorprothixene)[5].

Normal rhesus monkey erythrocytes were incubated with various membrane-active drugs (for 1 h at 37 degrees C) and after thorough washing, were exposed to infection with Plasmodium knowlesi in an invitro cultivation system.Marked inhibition of invasion was observed with vinblastine and colchicine, at concentrations greater than 5×10(-4) M, respectively. At similar concentrations, cytochalasin B or amantadine had no apparent effect. The addition of 10(-3) M 3',5'-cAMP to the medium during the incubation in the presence of colchicine or vinblastine partially decreased the inhibitory effects. The effects of colchicine and vinblastine on parasite invasion may be correlated with a reversible alteration in erythrocyte conformation (spherocytosis) which occurs at similar drug concentrations to those above and which can be relieved by simultaneous incubation with cAMP.[6] Because ferriprotoporphyrin IX (ferriheme, FP) is a lytic agent that can be released by degradation or oxidative denaturation of hemoglobin, measured the hemolytic responses of human erythrocytes to FP alone or to FP in combination with various membrane-active agents. Suspensions of erythrocytes (0.5%) incubated at pH 7.4 and 37 degrees C were hemolyzed by FP alone in concentrations of 10 microM or greater. Preincubation of the erythrocytes with nonhemolytic concentrations of chloroquine, mefloquine, quinine, calcium, lanthanum or manganese potentiated the hemolytic response to FP. For example, hemolysis in the presence of 5 microM FP was 5%; in the presence of 5 microM FP and 20 microM chloroquine, hemolysis exceeded 80%. For 5 microM FP, maximal potentiation was obtained with 20 microM chloroquine, 200 microM quinine or 1 mM calcium. Paradoxically, with 5 microM FP and a high concentration of chloroquine (1 mM), hemolysis did not exceed the base- line value of 5%. In addition, all of the agents that individually potentiated the hemolytic response to 5 microM FP also inhibited hemolysis when used in combination with 5 microM chloroquine and 5 microM FP[7].

To determine the efficacy of novel photoproducts in preactivated merocyanine

540 (pMC540) against human breast cancer cells (MCF-7) transplanted into athymic mice,treatment of serially propagated solid breast xenografts with pMC540 (250 mg/kg) caused a significant (43%) suppression (P = .004; P = .0882; P = .0903) in tumor area, weight, and volume, respectively. Treatment with tamoxifen alone was ineffective. However, a combination of tamoxifen and pMC540 (100 mg/kg) caused a 67% suppression of breast tumor growth. Treatment of implanted tumor with 20 doses (75 mg/kg) of merodantoin, a chemically synthesized photoproduct originally isolated from pMC540, suppressed the tumor growth by 98%. Photoproducts in pMC540 by themselves and in combination with tamoxifen are effective in suppressing the in vivo growth of transplanted human breast tumor[8].

Vancomycin,an antibiotic produced by *Amycolatopsis orientalis*,active only against gram-positive bacteria, particularly staphylococci. Vancomycin is a glycopeptide of molecular weight 1500, water-soluble and quite stable.Vancomycin inhibits cell wall synthesis by binding firmly to the D-Ala-D-Ala terminus of nascent peptidoglycan pentapeptide. This inhibits the transglycosylase, preventing further elongation of peptidoglycan and cross-linking. The peptidoglycan is thus weakened, and the cell becomes susceptible to lysis. The cell membrane is also damaged, which contributes to the antibacterial effect.

The antifungal activity and cytotoxicity of a novel membrane-active peptide, KKVVFKVKFKK (MP) which inhibited the growth of various pathogenic fungi isolated from patients and of fluconazole-resistant fungi at concentrations of 2 to 32 Âµg/ml. MP had potent fungicidal activity; the minimal fungicidal concentrations of the peptide were no more than fourfold greater than the MICs. Time course experiments of MP-induced killing of *Candida albicans* ATCC 36232 showed that the rate of killing was rapid and depended on the concentration of MP. MP had a strong synergism with other antifungal drugs; the fractional inhibitory concentration index values of MP with amphotericin B and fluconazole for *C. albicans* were 0.16 and 0.02, respectively. The 50% inhibitory concentrations of MP for NIH 3T3 and Jurkat cells were approximately 100 times higher than the MIC for *C. albicans* ATCC 36232, indicating that MP had high selectivity between the fungal and mammalian cells. MP has great advantages in the development of antifungal agents[9].

Bradykinin (1-100 nM) caused a reversible membrane potential depolarization of ganglion neurons that was not associated with a change in input resistance. The selective bradykinin B_2 receptor antagonist HOE-140 inhibited bradykinin-induced membrane depolarizations. Furthermore, the cyclooxygenase inhibitor indomethacin attenuated bradykinin-induced membrane depolarizations to a similar magnitude (~70%) as HOE-140. However, neurokinin-1 and -3 receptor antagonists did not have similar inhibitory effects. The ability of bradykinin to directly alter active properties of parasympathetic ganglion neurons was also examined. Bradykinin (100 nM) significantly reduced the duration of the afterhyperpolarization (AHP) that followed four consecutive action potentials. The inhibitory effect of bradykinin on the AHP response was reversed by HOE-140 but not by indomethacin[10].

The *Solanum nigrum* Linne (SNL) has been traditionally used as a herbal agent in folk medicine. SNL glycoprotein consists of carbohydrate (69.74%) and protein content (30.26%), which has mainly the hydrophobic amino acids containing glycine and proline. With respect to its characters, evaluated the apoptotic effects of glycoprotein isolated from SNL in human cervical cancer cell. In the activity of the apoptotic related proteins [cytochrome *c*, caspase 8, 3 and poly (ADP-ribose) polymerase (PARP)], the results showed that SNL glycoprotein (50 Î¼g/ml) has a stimulatory effect on cytochrome *c* release into cytosol, caspase 8, 3 activation and PARP cleavage in HeLa cells.The activities of NF-ÎºB and AP-1 significantly decreased after SNL glycoprotein (50 Î¼g/ml) treatment for 4 h, compare to the control. Interestingly, there was no difference of the DNA binding activity between NF-ÎºB and AP-1. Also, nitric oxide (NO) production was significantly declined at 50 Î¼g/ml SNL glycoprotein for 4 h. SNL glycoprotein exhibits inhibitory effect on HeLa cells via apoptosis, and it may be a potential candidate in field of anticancer drug discovery[11].

Trans fatty acids (TFA) can modify cellular function by interacting with hydrophobic regions of membrane proteins. Because these interactions resemble those of pharmacological agents, it might be appropriate to view TFA as drugs[12].

The participation of an Na^+ K^+ transport mechanism in glycolytic control is suggested by the decrease in glycolysis observed in the presence of ouabain and in medium devoid of potassium. ATP levels are conserved under both conditions. The involvement of ion transport in glycolytic control is also suggested by the effects of the membrane-active compound propranolol on motility and glycolysis. At 0.8 m*M* propranolol markedly inhibits the motility of washed sperm and causes about a 30% reduction in the glycolytic rate with a sharp fall in intracellular ATP, a rise in ADP, and an increase in triosephosphate. ATP loss is partially blocked by ouabain but ouabain does not prevent the loss of motility or the inhibition of glycolysis. The effects of propranolol on glycolysis, however, can be blocked by nucleotides. These compounds also partially restore motility to inhibited cells.[13]

A2780 and COLO-316 ovarian adenocarcinoma cell lines were exposed to 1.0 mM l -octanol for 12 hr in order to evaluate the potential effects of inhibition of gap junction-mediated intercellular communication (GJIC) on cellular responses to the chemotherapeutic drug melphalan.In cells which were sensitive to melphalan, octanol enhanced melphalan toxicity in the GJIC-competent (A2780/S) but not GJIC-incompetent (COLO-316/S) sensitive cells. Although octanol increases plasma membrane lipid mobility in A2780/S and COLO-316/S, it appears that enhancement of A2780/S sensitivity to melphalan may be due to inhibition of GJIC. In melphalan-resistant cells (A2780/R and COLO-316/R), 1.0 mM octanol treatment for 12 hr combined with melphalan reversed the resistance of the cells to the drug.[14]

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