Anti-Cancer Drugs, 3, pp. 443–453.

Review Paper

Cell membranes as targets for anti-cancer drug action

Sayed S Daoud
The author is at the Department of Pharmaceutical Sciences, Pharmacology and Toxicology Graduate Program, College of Pharmacy, Washington State University, Pullman, WA 99164-6510, USA.
Fax: (509) 335-0162.

A variety of studies strongly hint that cell membranes can be important targets for new and existing anti-tumor drugs. Traditionally, the search for new anti-cancer agents has focused on compounds designed to act on the biosynthesis, stability or function of DNA; it may be timely, however, to broaden our search horizon and consider targets outside of the nucleus. Thus, the cell membrane as well as membraneous organelles may well play a key role in the future of anti-tumor drug development.

Key words: Cell membranes, doxorubicin, ether lipids, immunotoxins, ionophores, liposomes.

Introduction

The plasma membrane, as well as the internal cellular membranes of the mitochondria, endoplasmic reticulum and golgi apparatus, play important roles in cell architecture and function. Thus they would seem to be appropriate targets for cytotoxic drugs to be used in cancer therapeutics; however, as is obvious from the dearth of anti-tumor agents which act at this level, cell membranes have been slighted as a target for drug development. The purpose of this brief review is to explore prospects and limitations concerning the discovery and development of anti-tumor drug candidates designed to act primarily upon cell membranes. There is substantial, but rather widely dispersed, literature on membrane actions of anti-tumor drugs. Without attempting an exhaustive review, this article will try to point out some of the themes in the recent literature on this topic and appraise how these themes might be further developed.

For most currently available anti-neoplastics, including nucleoside analogs, antifolates, alkylating agents and intercalators, the primary mechanism of cytotoxic action involves effects on the biosynthesis, stability or function of DNA.[1] This is certainly a reasonable site of action since DNA is clearly a critical target for the inhibition of neoplastic cell multiplication. The selectivity of these anti-cancer drugs is based on the exploitation of differences in DNA organization, replication and repair between normal and malignant cells. For example, altered uptake and polyglutamylation of folates in tumor cells may be responsible for the relative selectivity of methotrexate (MTX) and its analogs,[2] while efficient phosphorylation of cytosine arabinoside to form Ara CTP in certain leukemias may be the basis of the drug action.[3] However, events at the level of DNA synthesis and function are very fundamental and highly conserved; thus the biochemical alterations in neoplastic cells that permit the selective action of anti-cancer drugs are usually rather subtle and often poorly understood. Therefore, most agents which act on tumor cells via DNA also have profound toxicities to rapidly dividing normal cell populations. These toxicities, including bone marrow suppression, damage to the gastrointestinal epithelium and damage to skin, often limit the effectiveness of anti-cancer therapy.

In addition to processes involving DNA, events based in cell membranes are also critical for cell growth and replication. For example, the proton gradient across the mitochondrial membrane is essential for the generation of ATP in most cells.[4] Maintenance of an appropriate ion gradient across the plasma membrane is vital for protein synthesis,[5]

This work was supported by a grant from the Elsa U Pardee Foundation.

© *1992 Rapid Communications of Oxford Ltd*

for enzymes function[6] and for osmotic stability,[7] loss of which would lead to cell lysis and death.[8] Thus, drugs that act to damage membranes or to inhibit critical membrane processes might have considerable potential as cytotoxic anti-cancer agents (Figure 1). As in the case of events at the nuclear level, there are numerous potentially exploitable, albeit subtle, differences between normal and malignant cells in terms of membrane organization and function; some of these differences will be discussed immediately below. In thinking about membrane-active agents as anti-cancer drug candidates, however, one must keep their likely toxicities firmly in mind. Drugs of this type may profoundly impair cells and tissues which utilize trans-membrane ion gradients to serve vital functions such as communication (e.g. the nervous system), mechanical work (e.g. the heart) or excretion (e.g. the kidney). The specter of these very important acute toxicities has in the past, no doubt, dampened enthusiasm for rational development of membrane-active anti-tumor drugs and has likely impeded their emergence from drug screening programs. Nonetheless, it is important to note that the tissues affected by the toxicities of membrane-active drugs are likely to be different from those drugs which act via DNA, this remains to be fully documented. Thus, if the most severe toxicities of membrane-active drugs can be avoided, these agents may work very well in combination therapy with 'standard' anti-cancer drugs (those acting on DNA), because of non-overlapping tissue sites of dose-limiting toxicity.

Characteristics of membranes of normal versus malignant cells

Many investigators in the past decade have invested a great deal of effort looking for chemical, physical and functional differences between the plasma membranes or intracellular membranes of normal cells and their transformed or malignant counterparts.[9] For example, in the late 1970s and early 1980s several comprehensive review articles examined the abundant information which had been accumulated by that time.[10–13] The membranes of many transformed cells seemed to differ from those of most normal cells with respect to several different sets of parameters:

(i) *Glycosylation patterns.* Aberrant oligosaccharide compositions of glycolipids and/or glycoproteins have been observed for many tumor cells.[14] Often the tumor cell phenotype involves incomplete synthesis of membrane gangliosides and accumulation of precursors; however, many other types of altered glycosylation have been observed. Since glycolipids which contribute to membrane physical properties are also involved in cell interactions and regulation of receptor function, the altered patterns of glycosylation in tumor cells could be very important functionally.

(ii) *Membrane fluidity.* Metastatic properties of cells have been associated with differences in cell surface membrane fluidity and lipid composition.[15] This is probably best documented for certain lymphomas whose membranes seem more fluid than their normal lymphoid counterparts.[16,17] The altered

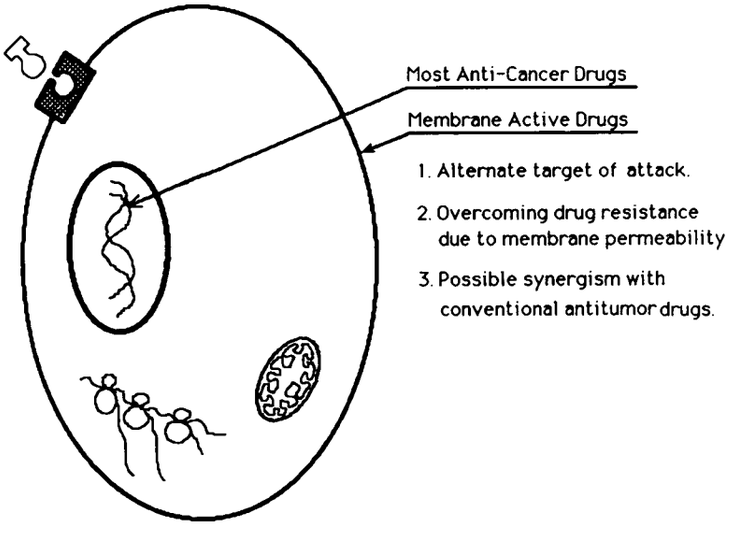

Figure 1. Cell membranes as an alternate target for anti-cancer drugs.

glycolipid composition often found in tumor cells may contribute to the observed changes in membrane fluidity. Cholesterol homeostasis in highly malignant cells may also be less regulated as compared with local tumors or in normal tissue.[18,19] The abnormal regulation of membrane cholesterol content, fatty acid composition, cholesterol/phospholipid ratio and feed back inhibition of cholesterol biosynthesis observed in highly malignant cells may be associated with differences in the function and/or quantity of a group of cytosolic lipid transfer proteins, i.e. the sterol carrier proteins (SCPs).[20]

(iii) *Membrane–cytoskeletal linkages.* One of the most dramatic and consistent differences noted between transformed cell lines and their non-transformed counterparts concerns enhanced agglutinability of lectins.[10,21] This is accompanied by an increased mobility of the glycoproteins which serve as 'lectin receptors'. The biochemical basis of this phenomenon is thought to be a weakened anchorage between cell surface glycoproteins and cytoskeletal elements subtending the plasma membrane. The general validity of this phenomenon has been shown to be true with members of the integrin super-family. A member of the cell surface adhesion receptors has been shown to display a diffuse distribution in transformed cells while being tightly anchored to cytoskeletal structures in focal contacts in non-transformed cells.[22,23] This rather fundamental alteration in the linkage of membrane proteins to the cytoskeleton in tumor cells may have important implications for cell adhesion, motility, invasiveness and metastatic ability,[24] as well as in terms of cellular responses to membrane-active agents.

(iv) *Mitochondrial membrane changes.* A variety of differences have been observed between the mitochondria of tumor cells and those of their normal counterparts.[25,26] These include alterations in mitochondrial abundance, morphology and utilization of substrates. Another common observation is that tumor mitochondria exhibit reduced 'acceptor control ratios', indicating that the inner mitochondrial membrane is leaky to protons; this may be an important factor for modulating cellular resistance of anti-cancer drugs such as cisplatin resistance in tumor cells.[27] Finally, in tumor cells there is often an altered balance between the Krebs cycle and glycolysis in terms of ATP production, with relatively more glycolysis and lactate production in cancer cells.

(v) *Other aspects.* There exist in the literature descriptions of many other differences between membranes of tumor cells and those of their counterparts. Certainly, altered lipid modification of proteins (myristoylation, plamitoylation and isoprenylation), lipid regulation of proteins (protein kinase C; PKC), lipid metabolism (phosopholipase A, C and D), RAS and GAP[28] should be mentioned.

For drug development, one need not identify a unique difference between normal and all malignant cells. Rather, it is sufficient to identify characteristics which discriminate cells of a particular tumor from most cells of the host; certainly, many of the parameters discussed above meet this criterion. Therefore, it seems worthwhile to reconsider the accumulation of information on tumor cell membranes which has accrued in the previous decade, to add to this some developing insights concerning key membrane enzymes such as members of the PKC family, and to use this information to explore the possibility of identifying and developing membrane-active anti-cancer drugs. Recently, this specific subject has been extensively reviewed by Tritton and Hickman.[29]

Membrane actions of 'conventional' anti-cancer drugs

Many agents which have their primary action at the DNA level also have important actions at the membrane level. For example, alkylating agents are known to affect a number of membrane functions[30] including inhibition of ion transport.[31] Nucleoside analogs have been reported to modify cell surfaces[32] and to act on lipid membranes.[33] Cisplatin has been reported to inhibit amino acid transport[34] and to cause alterations of membrane fluidity.[35]

The anthracyclines seem to interact with membranes in a variety of ways. Work by Tritton and his colleagues[36,37] and by Tokes' group,[38,39] using doxorubicin coupled to macromolecular carriers, showed that the drug could kill cells, presumably via membrane interactions, under conditions where no drug could reach the nucleus. It is also well known that the quinone moiety of anthracyclines can generate free radicals which lead to lipid peroxidation and membrane damage.[40] Lipid peroxidation, as well as the known propensity of doxorubicin to bind to the mitochondrial lipid cardiolipin, may be the underlying causes of the well known cardiomyopathy induced by high cumulative doses of doxorubicin[1] and may also be involved in

doxorubicin toxicity to tumor cells. In addition, more recent works by Tritton and co-workers[41,42] showed that adriamycin could increase the turnover rate of phosphotidylinositol (PI) in treated tumor cells and it appears that the disruption of this key early event in signal transduction may be involved in the cascade of events that mediates the cytotoxic action of adriamycin.

A new perspective on interactions between 'standard' anti-cancer drugs and membrane components has been provided recently by the work of Bell and his colleagues on PKC.[43] Cloning of the PKC gene has revealed the existence of a gene family encoding closely related isoforms.[44,45] The existence of a family of PKC polypeptides suggests that distinct PKC isoforms particpate in different cellular pathways, and seem to play a critical role in the transduction of membrane signals and in the regulation of cell growth.[46] However, recent findings indicated that alterations in the activity of PKC may be involved in malignant transformation processes.[47–49] PKC in its active form is a membrane-bound enzyme complexed with anionic lipids and calcium. PKC is activated by its endogenous ligand diacylglycerol and by phorbol esters,[46] it is inhibited by endogenous lipids such as sphingosine and lysophingolipids.[50,51] Interestingly, PKC is also inhibited by a number of useful or potential anti-tumor agents including the nucleoside analog sangivamycin, acridine derivatives, doxorubicin–iron(III) complex and 7-hydroxy staurosporine.[43,52–54] These findings suggest that PKC may be a potential target for cancer chemotherapy and may also provide an important new venue for the development of anti-tumor drug candidates which act on membrane-associated kinases.

Agents which enhance membrane permeation of anti-cancer drugs

Transport of an anti-tumor drug across the plasma membrane and its accumulation in cells is an important determinant of drug action. This has recently been emphasized in the context of the multi-drug resistance (MDR) phenomenon.[55–57] The most common form of MDR is due to over expression of the P-glycoprotein, a rather promiscuous ATP driven membrane efflux pump which can export a variety of amphiphilic cationic drugs from cells, including important anti-tumor agents such as vinca alkaloids and anthracyclines. Inhibition of the efflux function of the P-glycoprotein should permit increased accumulation of the anti-tumor drug and reversal of the drug resistant phenotype. Accordingly, considerable effort has recently gone into the search for non-cytotoxic agents which can inhibit the P-glycoprotein. A number of agents including verapamil and its analogs, cyclosporins, reserpine analogs, and other compounds may serve this purpose,[58–60] while structure–activity studies and molecular modeling are leading to an understanding of the characteristics of drug recognition by the P-glycoprotein.[61] The fact that a specific transport system is involved makes it most likely that highly selective agents can be designed to affect the drug efflux function of the P-glycoprotein without generalized membrane damage.

In addition to the MDR phenomenon, poor cellular accumulation of anti-tumor drugs can limit therapy in a variety of situations, as in the case of a tumor population resistant to cisplatin therapy.[62,63] Accordingly, investigators have examined the possibility that membrane-disrupting agents such as cyclosporin A, valinomycin and lonidamine[64–66] might potentiate cisplatin cytotoxicity. The basis of this effect is not clear at this time, but these agents are known to have significant effects on the mitochondria, thus the mitochondrial membrane may hold important keys for modulating cisplatin cytotoxicity in tumor cells. In any case, the synthesis of relatively non-toxic agents which act on membrane transport systems or on the innate structure of the lipid bilayer, in a manner which potentiates the sensitivity of 'standard' anti-cancer drugs, certainly seems a worthwhile goal for drug development.

Potential anti-tumor agents which act on cell membranes

Phospholipid analogs as anti-tumor drugs

In the mid-1960s, Munder and colleagues discovered that lysolecithin analogs with an ether linkage at position 1 of glycerol exhibited interesting cytotoxic activities against tumor cells.[67] Somewhat later, Hanahan and co-workers showed that lipids of similar structure were the basis of platelet activating factor (PAF), a potent biological mediator.[68] These findings have led to a vast amount of work on the biological actions of lysophospholipids with ether or other poorly hydrolyzable linkages at the 1 position and, in

particular, to many explorations of their potential role in tumor therapy.

Some of the anti-tumor actions of the ether or alkyl lysophospholipids seem to be due to direct cytotoxic actions on tumor cells,[69,70] enhancement of the tumoricidal capacity of macrophages[71] or induction of cell differentiation.[72] Ether lipids and similar analogs have been shown to be powerful inhibitors of PKC;[73,74] however, other membrane enzymes such as Na/K ATPase may also be affected.[75] Ether lipids also may have direct effects on the structure of the lipid bilayer and may cause changes in fluidity,[76,77] and/or membrane permeabilization and disruption.[78] In addition to direct actions on tumor cells, some lysophospholipid derivatives may also act via stimulation of the immune system[79]—this may be especially true for analogs with PAF-type activities—and also show promising results in purging leukemia cells from bone marrow *in vitro*.[80]

While these findings are quite interesting, clinical therapeutic experience thus far with first generation ether lysophospholipids has been somewhat encouraging.[81,82] However, more sophisticated investigations into the actions of these agents on the lipid bilayer and on key membrane enzymes and receptors in various types of cancer cells should hopefully lead to the design of more potent and specific analogs.

Ionophores as anti-tumor drugs

An interesting and important group of compounds which act on cell membranes are the agents known as ionophores. This designation does not connote any common chemical structure, but rather a common functionality. Ionophores are a heterogeneous set of compounds which have the ability to increase membrane permeability towards mono- or divalent cations or other small molecules.[83,84] Mobile ionophores bind metal ions or organic cations to form lipid soluble complexes which can then shuttle back and forth across the cell membrane. Good examples of this type are valinomycin, a proton and potassium selective cyclic depsipeptide, and the carboxylic ionophores monensin and nigericin. Channel forming ionophores create water filled transmembrane channels or pores of narrow diameter which permit the movement of ions and small molecules; examples of this type include gramacidin and amphotericin B. Ionophore induced fluxes can disrupt a multitude of cellular functions including osmotic stability, protein synthesis and energy metabolism,[6,85,86] and thus ionophores can exert powerful cytotoxic actions. Obviously, cells of organs and tissues which rely on ion gradients for their function (e.g. nerve cells, heart cells) might be particularly susceptible to the cytotoxic effects of ionophores. At the present time very few ionophoric compounds are used therapeutically, largely because of their potential for cardiotoxicity and nephrotoxicity. The only ionophore used in the clinic for systemic therapy is amphotericin B, a compound valuable in the treatment of disseminated fungal infections.[87] Nonetheless, ionophores may have potential utility in the control of neoplasia, both as direct cytotoxic agents and as modulators of the actions of other anti-tumor drugs. It is this premise which we have begun to explore over the past several years.

We have made some progress in learning to use ionophores as experimental anti-tumor drugs, concentrating on the agent valinomycin, which had previously been reported to display anti-neoplastic activity *in vivo*.[88] A main concern of our early investigations was finding a way to mitigate the expected neuro- and nephrotoxicity of valinomycin and other ionophores. We found that we could reduce or 'buffer' the host toxicity of valinomycin by use of a lipid vesicle based drug delivery system (liposome) (Figure 2). This could be done while maintaining or enhancing the antitumor activity of valinomycin as assessed in the P388 mouse leukemia system (Figure 3).[89] The basis of this effect is not entirely clear, but may relate to an altered organ/tissue distribution of the liposomal valinomycin which diverts the drug from key sites of toxicity such as the cardiovascular system.[90,91] The enhancement of the therapeutic index of valinomycin by its incorporation into liposomes was paralleled by reduced toxicity to 'normal' cells *in vitro* and enhanced selectivity to c-Ha-*ras*-transformed cells.[92] It is possible that the *ras*-transformed cells have a higher endocytotic capability than the 'normal' cells and thus tend to accumulate liposomal valinomycin. Alternatively, there may be difference in the plasma membrane lipid characteristics of 3T3 and Ha-*ras*/3T3 cells that tends to favor the partitioning of liposomal valinomycin into the membranes of the transformed cells. We are currently pursuing these questions, trying to understand the innate anti-tumor action of valinomycin on 'normal' and oncogene transformed cells.

We also began to investigate possible synergisms between valinomycin and established 'standard'

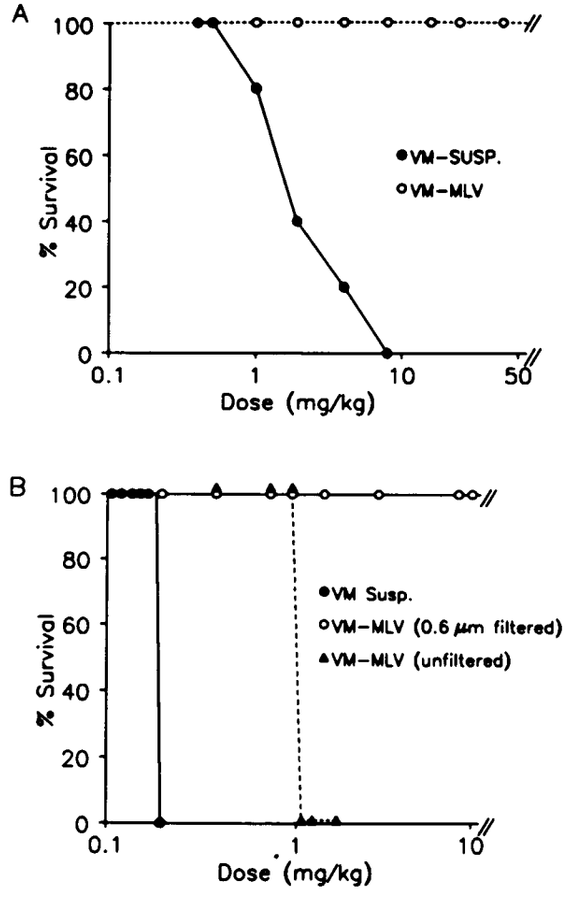

Figure 2. Toxicity of free or liposomal valinomycin in mice. Various doses of free drug or drug in phosphatidyl choline/phosphatidyl serine/cholesterol liposomes were administered i.p. or i.v. to healthy $B_6D_2F_1$ mice (six mice per group). Survival was evaluated over a 30 day period. Liposomes were either multilamellar vesicles without further processing or were filtered through 0.6 mm Nucleopore filters.

anti-tumor drugs, i.e. those acting primarily on DNA. An important set of experiments showed that low doses of valinomycin could enhance cisplatin cytotoxicity in fibroblastic cells[93] as well as epithelial tumor cells[66] *in vitro*. The potentiating effect of liposomal valinomycin on cisplatin cytotoxicity raised the question whether this phenomenon is additive or synergistic. To address this question, we assessed the outcome of the drug combination using the median-effect principle[94] that clearly showed synergistic cytotoxic effects for the combination of cisplatin and liposomal valinomycin in human tumor cells (Figure 4). The mechanism underlying this synergistic interaction is currently unknown. One possible explanation would be that valinomycin increased the cellular uptake of cisplatin, leading to a consequent increase in the formation of platinum–DNA adducts. From Figure 5, it is clear that only a modest increase in cell-associated platinum occurred on simultaneous treatment with cisplatin and valinomycin. Thus, it does not appear that the effects of valinomycin on cisplatin accumulation are sufficient to fully explain the cytotoxic synergism. Recently, we reported[95] that valinomycin blocks the progression of cells at the G_2 phase, i.e. the sensitive phase for cisplatin action.[96] This could be one basis for the cytotoxic synergism observed between cisplatin and valinomycin. Alternatively, valinomycin may discharge the pH gradient across the membranes of the endosome/lysosome compartment which will affect the cytoplasmic pH and, conseqently, tumor cell proliferation. This could lead to changes in the rate or magnitude of the chemical and enzymatic processes that are responsible for cisplatin-induced DNA damage or repair. All these processes are being actively explored in our laboratory.

These initial observations suggest that ionophores can exhibit anti-tumor efficacy as individual agents. In addition, one may be able to use valinomycin (or other ionophores), especially in a suitable drug delivery system like liposomes, to enhance the anti-tumor actions of established drugs such as anthracyclines or cisplatin, possibly at a minimal cost in additional host toxicity. It is interesting to note that investigators studying the effect of other ionophores on cells have described results and approaches very similar to those obtained with valinomycin by our laboratory. For example, Marks and colleagues[97] recently showed that the carboxylic ionophore monensin can potentiate the cytotoxic action of immunotoxin, both *in vitro* and *in vivo*. Similar results obtained by Griffin and Raso[98] have also indicated that monensin, when delivered in emulsion form, can indeed potentiate immunotoxin effects against human colorectal carcinoma and mesothelioma. The mechanism by which monensin potentiates the action of immunotoxins is not yet clear. Nigericin, another carboxylic ionophore, has recently been reported to enhance the cytotoxic effects of mafosfamide (a precursor of activated cyclophosphamide) *in vitro*.[99] The effect of nigericin, in this study, probably relates to the cellular modulation of pH_i which enhances the H^+ ion-mediated potentiation of the cytotoxicity of the alkylating drug. The modulation of the anti-tumor actions of established anti-neoplastics by these ionophores is exactly the goal of the cancer chemotherapist;

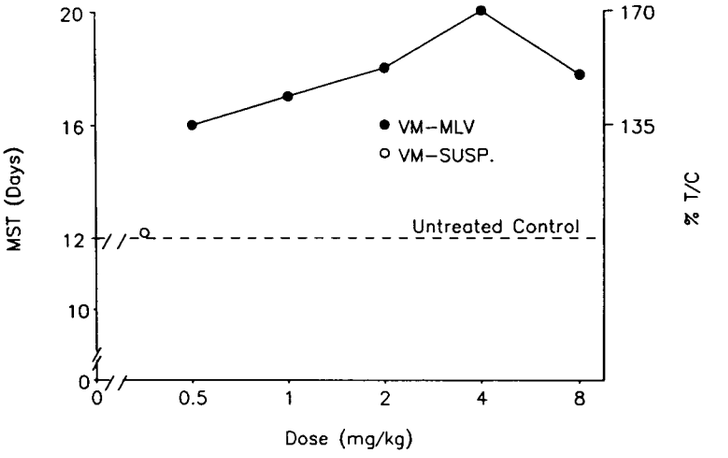

Figure 3. Anti-tumor effects of liposomal valinomycin. B_6DF_1 mice were inoculated i.p. on day 0 with 1×10^6 P388 leukemia cells. Mice were then treated i.p. on day 1 with various doses of free or liposome-incorporated valinomycin. Groups of six mice were used for each point. Survival was monitored over 30 days. Results are expressed as increased lifespan of treated over control (%T/C).

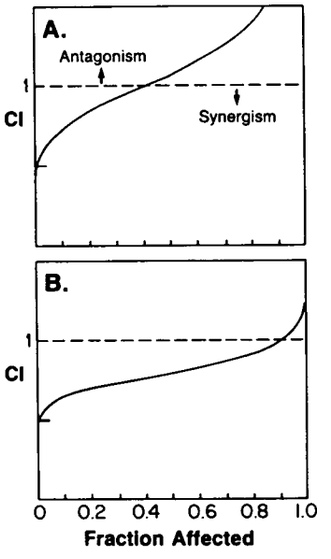

Figure 4. Combined effect of 3 h (A) and 72 h (B) exposure of CaOV-3 human ovarian carcinoma cells to cisplatin plus liposomal valinomycin. Computer-generated curves describe the combined effects of cisplatin plus liposomal valinomycin at fixed ratios. Results are plotted as a function of the fraction of treated cells affected versus the combination index (fa–CI plot) under a mutually non-exclusive assumption. All points lying above a CI of 1 are antagonistic, those lying below a CI of 1 are synergistic and those lying at a CI of 1 are additive. Interaction of cisplatin and liposome-incorporated valinomycin is strongly synergistic over nearly the entire range of concentrations tested (B), whereas it is antagonistic over the higher concentrations of drugs tested and synergistic at lower concentrations (A).

however, the molecular basis for such modulation should be exploited and determined.

Conclusions and perspective

Two major themes emerge from consideration of recent studies on the interplay between anti-tumor drugs and cell membranes. First, it has become clear that the 'standard' anti-cancer drugs, once thought to act primarily at the DNA level, may also have major effects on membranes. For example, anthracyclines (adriamycin–iron(III) complex) turn out to be potent inhibitors of PKC.[42,43] The transduction of signals from several growth factors and hormones impinge upon PKC; thus this family of membrane-associated enzymes plays a critical role in the control of cell growth and differentiation. This raises the question of whether many of the observed growth inhibitory effects of anthracyclines, like adriamycin–iron(III) complex and, perhaps, of other lipophilic cationic intercalators as well, may be medated by actions on critical membrane enzymes such as PKC. It may then be possible to synthesize analogs of intercalators while optimizing interactions with membranes with minimum DNA binding; these analogs may have a novel spectrum of therapeutic and toxic properties complementing those of existing drugs.

A second theme is that agents which act primarily upon cell membranes can be potent anti-tumor

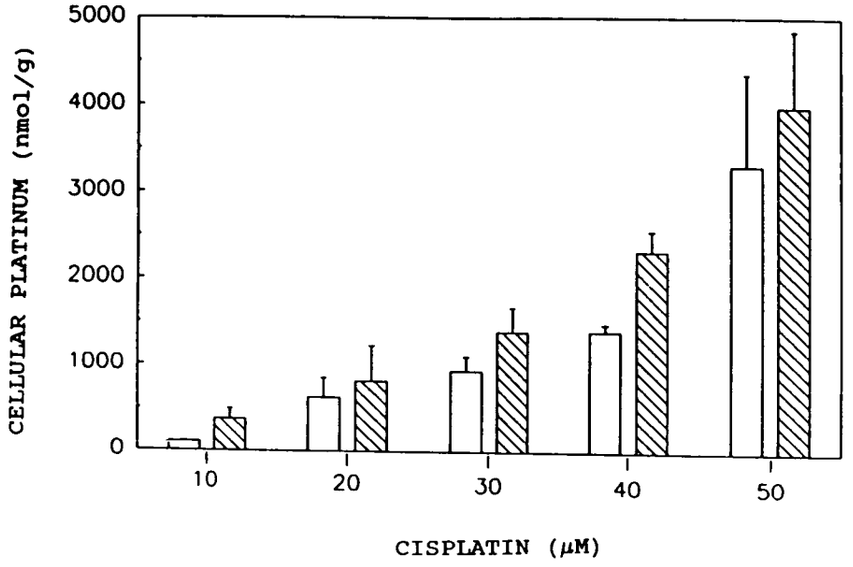

Figure 5. Accumulation versus concentration of cisplatin. The cell associated platinum content is shown as measured by flameless atomic absorption spectroscopy of acid extracts of CaOV-3 human ovarian carcinoma cells treated for 3 h with either cisplatin alone (□) or in combination with 20 nM valinomycin (▨). Bars represent the mean (±SE) of three experiments.

agents in their own right and can exhibit synergistic effects with 'standard' anti-cancer drugs. Thus, for ionophores and for ether lysophospholipids, which both display substantial anti-tumor activity, structural, biochemical and cell biological considerations all suggest that the primary action of these agents is on cellular membranes. Based on these observations, the synthesis of new anti-tumor drug candidates designed to act specifically on cell membranes would seem a worthwhile approach. This approach would be facilitated, however, if we knew more about the mechanism(s) of action of existing membrane-active drugs such as valinomycin or the ether lipids.

Several possible mechanisms can be suggested for the observed actions of ether lipids and ionophores on cells. Thus, the cytotoxic effects of these agents may be due to inhibition of critical membrane enzymes such as PKC. This is certainly a valid possibility for the ether lysophospholipids,[73–75] but has not been explored for ionophores. Physical perturbation of membranes, including changes in lipid fluidity, permeability or overall mechanical stability, may also be important aspects of the actions of these compounds.[76–78] Ionophores may have some unique effects on cells; thus even very low concentrations of ionophores can perturb ion gradients which are critical for cell function. For example, valinomycin is known to perturb the mitochondrial membrane potential,[4,86] and thus disrupt the connection between electron transport and oxidative phosphorylation. Other ionophores impair the cell's ability to maintain cytoplasmic pH, a critical parameter for cell growth,[100] or can disrupt the orderly recycling of membrane proteins.[101] A very interesting set of observations concerns the interplay between 'membrane-active' and 'standard' or 'conventional' anti-tumor drugs (e.g. anthracyclines, alkylating agents or platinum compounds). Ionophores have been shown to potentiate the action of immunotoxins or established anti-tumor agents,[66,94,98–100] especially in diseases confined to the peritoneal cavity. While it is clearly premature to state that membrane-active drugs generally sensitize cells to agents which act on DNA, the results obtained thus far are suggestive and promising.

Acknowledgments

We thank Dr Thomas R Tritton, Department of Pharmacology, University of Vermont, for helpful comments. The author would like to thank Jackie Bair for her expert typing of the manuscript.

References

1. Daskal Y. Drug effects on nuclear and extranuclear chromatin. In: Busch H, Crooke ST, Baskal Y, eds. *Effects of drugs on the cell nucleus.* New York: Academic Press 1979: 107–24.
2. Sirotnak, FM. Concepts in new folate analog design. In: Cheng YC, Goz B, Minkoff M, eds. *Progress in cancer research and therapy.* New York: Raven Press 1983: 77–88.
3. Rustum YM, Preisler HD. Correlation between leukemic retention of 1β-D-arabinofuranosyl cytosine-5′-triphosphate and response to therapy. *Cancer Res* 1979; **39**: 42–9.
4. Mandel LJ. Bioenergetics of membrane transport processes. In: Andreoli T, ed. *Physiology of membrane disorders.* New York: Plenum Medical 1986; 295–307.
5. Shibuya ML, Buddenbaum WE, Don AL, *et al.* Amsacrine-induced lesions in DNA and their modulation by novobiocin and 2,4-dinitrophenol. *Cancer Res* 1991; **51**: 573–80.
6. Breitbart TH, Herzog M. Membrane mediated inhibition of protein synthesis by valinomycin in reticulocytes. *FEBS Lett* 1973; **32**: 15–8.
7. Hoffman JF. Active transport of Na^+ and K^+ by red blood cells. In: Andreoli T, ed. *Physiology of membrane disorders.* New York: Plenum Medical 1986: 221–31.
8. Teicher BA, Herman TS, Holden SA, *et al.* Lonidamine as a modulator of alkylating agent activity *in vitro* and *in vivo. Cancer Res* 1991; **51**: 780–4.
9. Hynes RO. Tumorigenicity, transformation and cell surfaces. In: Hynes RO, ed. *Surfaces of normal and malignant cells.* New York: John Wiley 1979: 1–21.
10. Nicolson GL. Transmembrane control of the receptors on normal and tumor cells and surface changes associated with transformation and malignancy. *Biochim Biophys Acta* 1976; **458**: 1–72.
11. Nicolson GL. Cancer metastasis: organ colonization and the cell surface-properties of malignant cells. *Biochem Biophys Acta* 1982; **695**: 113–76.
12. Wallach DF. Membrane biology and cancer therapy. *Pathobiol Ann* 1978; **8**: 189–216.
13. Pederson PL. Tumor mitochondria and the bioenergetics of cancer cells. *Prog Exp Tumor Res* 1978; **22**: 190–74.
14. Hakomori SI. Aberrant glycosylation in cancer membranes as focused on glycolipids: a review and perspective. *Cancer Res* 1985; **45**: 2405–14.
15. Kier AB. Membrane properties of metastatic and non-metastatic cells cultured from C_3H mice injected with LM fibroblasts. *Biochim Biophys Acta* 1990; **1022**: 365–372.
16. Inbar M, Schinitsky M. Increase of cholesterol levels in the surface membranes of lymphoma cells and its inhibitory effect on ascites tumor development. *Proc Natl Acad Sci USA* 1974; **71**: 2128–32.
17. Van Blitterswijk WJ. Alterations in lipid fluidity in the plasma membrane of tumor cells. In: Schinitsky M, ed. *Physiology of membrane fluidity.* London: CRC Press 1986: 53–84.
18. Wallach DFH. *Membrane molecular biology of neoplastic cells.* Amsterdam: Elsevier 1975: 217–41.
19. Chen HW, Kandutsch AA, Heiniger HJ. The role of cholesterol in malignancy. *Prog Exp Tumor Res* 1978; **22**: 275–316.
20. Shroeder F, Kier AB, Olson CD, *et al.* Correlation of tumor metastasis with sterol carrier protein and plasma membrane sterol level. *Biochem Biophys Res Commun* 1984; **124**: 283–9.
21. Nicolson, GL. Organ colonization and the cell-surface properties of malignant cells. *Biochim Biophys Acta* 1982; **695**: 113–76.
22. Juliano, RL. Membrane receptors for extracellular matrix macromolecules: relationship to cell adhesion and tumor metastasis. *Biochim Biophys Acta* 1987; **907**: 261–78.
23. Duband JL, Nuckolls GH, Ishihara A, *et al.* Fibronectin receptor exhibits high lateral mobility in embryonic locomoting cells but is immobile in focal contacts and fibrillar streaks of stationary cells. *J Cell Biol* 1988; **107**: 1385–96.
24. Saiki I, Iida J, Murata J, *et al.* Inhibition of the metastasis of murine malignant melanoma by synthetic polymeric peptides containing core sequences of cell-adhesive molecules. *Cancer Res* 1989; **49**: 3815–22.
25. Chen LB, Summerhayes IC, Nadakavukaren KK, *et al.* Mitochondria in tumor cells: effects of cytoskeleton on distribution and as targets for selective killing. *Cancer Cells* 1984; **1**: 75–86.
26. Chen LB. Mitochondria as a reservoir for anticarcinoma drugs. In: Tapiero H, Robert J, Lampidis TJ, eds. *Anticancer drugs.* London: John Libbey Eurotext 1989: 21–8.
27. Andrews PA, Albright KD. Mitochondrial defects in *cis*-diamminedichloroplatinum(II)-resistant human ovarian carcinoma cells. *Cancer Res* 1992; **52**: 1895–901.
28. Ashendel CL. Membrane lipid and signaling in cell growth regulation. In: Steele VE, Stoner GD, Boone CW, *et al.* eds. *Cellular and molecular targets for chemoprevention.* Boca Raton, FL: CRC Press 1992: 19–38.
29. Tritton TR, Hickman JA. How to kill cancer cell: membranes and cell signaling as targets in cancer chemotherapy. *Cancer Cells* 1990; **2**: 95–105.
30. Grunicke H, Doppler W, Hofmann J. Plasma membrane as a target for alkylating agents. *Adv Enzyme Reg* 1985; **24**: 247–261.
31. Doppler W, Hoffman J, Oberhuber H, *et al.* Nitrogen mustard interference with potassium transport systems in Ehrlich ascites tumor cells. *J Cancer Res Clin Oncol* 1985; **110**: 35–41.
32. Kessel D. Cell surface alterations associated with exposure of L1210 leukemia cells to fluorouracil. *Cancer Res* 1980; **40**: 322–4.
33. Koehler KA, Hines J, Mansour EG. Comparison of the membrane related effects of cytarabine and other agents on model membranes. *Biochem Pharmacol* 1979; **28**: 583–8.
34. Scanlon KJ. Saffirstein RL, Thies H. Inhibition of amino acid transport by *cis*-diamminedichloroplatinum(II) derivatives in L1210 murine leukemia cells. *Cancer Res* 1983; **43**: 4211–5.
35. Simpkins H, Pearlman LF. Interaction of two second generation platinum antitumor drugs with mouse thymocytes. *Cancer Res* 1986; **46**: 1433–9.
36. Tritton TR, Yee G, Wingard Jr, LB. Immobilized adriamycin: a tool for separating cells surface from intracellular mechanisms. *FASEB J* 1983; **42**: 284–7.
37. Siegfried JA, Kennedy KA, Sartorelli AC, *et al.* The role of membranes in the mechanism of action of the antineoplastic agent adriamycin. Spin-labeling studies with chronically hypoxic and drug-resistant tumor cells. *J Biol Chem* 1983; **258**: 339–43.
38. Tokes ZA, Rogers KE, Rembaum A. Synthesis of

adriamycin-coupled polyglutaraldehyde microspheres and evaluation of their cytostatic activity. *Proc Natl Acad Sci USA* 1982; **79**: 2026–30.
39. Rogers KE, Carr BI, Tokes ZA. Cell surface mediated cytotoxicity of polymer-bound adriamycin against drug resistant hepatocytes. *Cancer Res* 1983; **43**: 2741–8.
40. Myers CE, McGuire WP, Liss RH, *et al.* Adriamycin: the role of lipid peroxidation in cardiac toxicity and tumor response. *Science* 1977; **197**: 165–8.
41. Tritton TR, Posada J. The role of the cell surface membrane in adriamycin resistance. In: Kessel D, ed. *Resistance to antineoplastic drugs*. London: CRC Press 1989: 127–40.
42. Posada J, Vichi P, Tritton TR. Protein kinase C in adriamycin action and resistance in mouse sarcoma 180 cells. *Cancer Res* 1989; **49**: 6634–39.
43. Hannum YA, Foglesong RJ, Bell RM. The adriamycin--iron(III) complex is a potent inhibitor of protein kinase C. *J Biol Chem* 1989; **264**: 9960–6.
44. Ono Y, Fujii T, Ogata K, *et al.* The structure, expression, and properties of additional members of the protein kinase C family. *J Biol Chem* 1988; **263**: 6927–32.
45. Ohno S, Kawasaki H, Imajoh S, *et al.* Tissue specific expression of three distinct types of rabbit protein kinase C. *Nature* 1987; **325**: 161–6.
46. Nishizuka Y. The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature* 1984; **308**: 693–8.
47. Persons DA, Wilkison WO, Bell RM, *et al.* Altered growth regulation and enhanced tumorigenicity of NIH 3T3 fibroblasts transfected with protein kinase C-1 cDNA. *Cell* 1988; **52**: 447–58.
48. Housey GM, Johnson MD, Hsiao HLW, *et al.* Overproduction of protein kinase C causes disordered growth control in rat fibroblasts. *Cell* 1988; **52**: 343–54.
49. Megidish T, Mazurek N. A mutant protein kinase C that can transform fibroblasts. *Nature* 1989; **342**: 807–11.
50. Hannum YA, Bell RM. Lysosphingolipids inhibit protein kinase C: implications for the sphinglipidoses. *Science* 1987; **235**: 670–4.
51. Hannum YA, Greenberg CS, Bell RM. Sphingosine inhibition of agonist-dependent secretion and activation of human platelets implies that protein kinase C is a necessary and common event of the signal transduction pathways. *J Biol Chem* 1987; **262**: 13620–6.
52. Hannum YA, Bell RM. Aminoacridines, potent inhibitors of protein kinase C. *J Biol Chem* 1988; **263**: 5124–31.
53. Loomis CR, Bell RM. Sangivamycin, a nucleoside analogue is a potent inhibitor of protein kinase C. *J Biol Chem* 1988; **263**: 1682–92.
54. Akinaga S, Gomi K, Morimoto M, *et al.* Antitumor activity of UCN-01, a selective inhibitor of protein kinase C, in murine and human tumor models. *Cancer Res* 1991; **51**: 4888–92.
55. Bradley G, Juranka PF, Ling V. Mechanism of multidrug resistance. *Biochim Biophys Acta* 1988; **948**: 87–128.
56. Beck WT. The cell biology of multiple drug resistance. *Biochem Pharmacol* 1987; **18**: 2879–87.
57. Pastan I, Gottesman M. Multiple drug resistance in human cancer. *New Engl J Med* 1987; **316**: 1388–93.
58. Tsuruo T, Iida H, Tsnkagoshi S, *et al.* Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Res* 1981; **41**: 1967–72.
59. Inaba M, Fujikura R, Tsukagoshi S, *et al.* Restored *in vitro* sensitivity of adriamycin and vincristine-resistant P388 leukemia with reserpine. *Biochem Pharmacol* 1981; **30**: 2191–184.
60. Twentyman PR. Modification of cytotoxic drug resistance by non-immunosuppressive cyclosporins. *Br J Cancer* 1988; **57**: 254–8.
61. Pearce HL, Safa AR, Bach NJ, *et al.* Essential features of the P-glycoprotein pharmacophore as defined by a series of reserpine analogs that modulate drug resistance. *Proc Natl Acad Sci USA* 1989; **86**: 5128–32.
62. Andrews PA, Velury S, Mann SC, *et al. cis*-diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Cancer Res* 1988; **48**: 68–73.
63. Andrews PA, Mann SC, Huynh HH, *et al.* Role of Na^+/K^+-adenosine triphosphatase in the accumulation of *cis*-diamminedichloroplatinum(II) in human ovarian carcinoma cells. *Cancer Res* 1991; **51**: 3677–81.
64. Kashani-Sabet M, Wang W, Scanlon KJ. Cyclosporin A suppresses cisplatin-induced c-*fos* gene expression in ovarian carcinoma cells. *J Biol Chem* 1990; **265**: 11285–8.
65. Raaphorst GP, Feeley MM, Heller DP, *et al.* Lonidamine can enhance the cytotoxic effect of cisplatin in human tumor cells and rodent cells. *Anticancer Res* 1990; **10**: 923–8.
66. Daoud SS, Forde NH. Synergistic cytotoxic actions of cisplatin and liposomal valinomycin on human ovarian carcinoma cells. *Cancer Chemother Pharmacol* 1991; **28**: 370–6.
67. Munder PG, Modolell M, Ferber E, *et al.* Phospholipids in quartz damaged macrophages. *Biochemistry* 1966; **344**: 31013–6.
68. Demopolous CA, Pinkert RN, Hanahan DJ. Platelet activating factor. Evidence for 1-*o*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphocholine as the active component for a new class of lipid chemical mediators. *J Biol Chem* 1979; **254**: 9355–8.
69. Andreesen R. Ether lipids in the therapy of cancer. *Prog Biochem Pharmacol* 1988; **22**: 118–31.
70. Bazill GW, Dexter TM. An antagonist to platelet activating factor counteracts the tumoricidal action of alkyl lysophospholipids. *Biochem Pharmacol* 1989; **38**: 374–7.
71. Yamamoto N, Ngwenya BZ, Sery TW, *et al.* Activation of macrophages by ether analogues of lysophospholipids. *Cancer Immunol Immunother* 1987; **25**: 185–92.
72. Honma Y, Kasukabe T, Hozumi M, *et al.* Induction of differentiation of cultured human and mouse myeloid leukemia cells by alkyl lysophospholipids. *Cancer Res* 1981; **41**: 3211–6.
73. Shoji M, Raynor RL, Berdel WE, *et al.* Effects of thioether phospholipid BM 41.440 on protein kinase C and phorbol ester-induced differentiation of human leukemia HL60 and KG-1 cells. *Cancer Res* 1988; **48**: 6669–73.
74. Hofman J, Ueberall F, Posch L, *et al.* Synergistic enhancement of the antineoplastic activity of cis-diamminedichloroplatinum(II) by the ether lipid analogue BM41440, an inhibitor of protein kinase C. *Lipids* 1989; **24**: 312–7.
75. Oishi K, Zheng B, White JF, *et al.* Inhibition of Na/K-ATPase and sodium pump by anticancer ether lipids and protein kinase C inhibitors ET-18-OCH_3 and BM 41.440. *Biochem Biophys Res Commun* 1988; **157**: 1000–6.

76. Neseda A, Godwin PL, Modest EJ. Effects of antineoplastic ether lipids on model and biological membranes. *Biochim Biophys Acta* 1988; **945**: 92–100.
77. Van Blitterswijk WJ, Hikmann H, Storme GA. Accumulation of an alkyl lysophospholipid in tumor cell membranes affects membrane fluidity and tumor cell invasion. *Lipids* 1987; **22**: 820–3.
78. Noseda A, White JG, Godwin PL, *et al.* Membrane damage in leukemic cells induced by ether and ester lipids: an electron microscopic study. *Exp Mol Pathol* 1989; **50**: 69–83.
79. Kudo I, Nojima S, Wook C, *et al.* Antitumor activity of synthetic alkylphospholipids with or without paf activity. *Lipids* 1987; **22**: 862–7.
80. Okamoto S, Olson AC, Berdel WE, *et al.* Purging of acute myeloid leukemia cells by ether lipids and hyperthermia. *Blood* 1988; **72**: 1777–83.
81. Berdel W, Fink U, Rastetter J. Clinical phase I pilot study of the alkyl lysophospholipid derivative ET-18-OCH_3. *Lipids* 1987; **22**: 967–9.
82. Andreesen R. Ether lipids in the therapy of cancer. *Prog Biochem Pharmacol* 1988; **22**: 118–31.
83. Ouchinnikov YA. Physico-chemical basis of ion transport through biological membranes: ionophores and ion channels. *Eur J Biochem* 1979; **94**: 321–36.
84. Pressman BL, Fahim M. Pharmacology and toxicology of the monovalent carboxylic ionophores. *Annu Rev Pharm Toxicol* 1982; **22**: 465–90.
85. Felber SM, Brand MD. Valinomycin can depolarize mitochondria in intact lymphocytes without increasing plasma membrane potassium fluxes. *FEBS Lett* 1982; **150**: 122–4.
86. Yannish K. Effects of valinomycin on hexose transport and cellular ATP pools in mouse fibroblasts. *J Cell Physiol* 1984; **119**: 163–71.
87. Franklin TJ, Snow GA. *Biochemistry of antimicrobial action.* London: Chapman & Hall 1981: 58–78.
88. Suffness M, Douris J. Miscellaneous natural products with antitumor activity. *Med Chem* 1980; **16**: 465–87.
89. Daoud SS, Juliano RL. Reduced toxicity and enhanced antitumor effects in mice of the ionophoric drug valinomycin when incorporated in liposomes. *Cancer Res* 1986; **46**: 5518–23.
90. Daoud SS, Hume LR, Juliano RL. Liposomes in cancer therapy. *Advanced Drug Deliv Rev* 1989; **3**: 405–16.
91. Juliano RL, Daoud SS, Krause H, *et al.* Membrane-to-membrane transfer of lipophilic drugs used against cancer or infectious disease. *Ann NY Acad Sci* 1987; **507**: 89–103.
92. Daoud SS, Juliano RL. *In vitro* effect of liposome-incorporated valinomycin on growth and macromolecular synthesis of normal and *ras*-transformed 3T3 cells. *Cancer Chemother Pharmacol* 1989; **23**: 151–5.
93. Daoud SS, Juliano RL. Modulation of doxorubicin resistance by valinomycin (NSC 122023) and liposomal valinomycin in chinese hamster ovary cells. *Cancer Res* 1989; **49**: 2661–7.
94. Chou T-C, Talalay P. Quantitative analysis of dose–effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Reg* 1984; **22**: 27–43.
95. Sakata MK, Daoud SS. Effects of liposome-incorporated valinomycin in combination with cisplatin and other analogues in human ovarian cancer. *Proc Am Ass Cancer Res* 1991; **32**: 2042.
96. Sorenso CM, Eastman A. Mechanism of *cis*-diamminedichloroplatinum(II)-induced cytotoxicity. Role of G_2 arrest and DNA double-stand breaks. *Cancer Res* 1998; **48**: 4484–8.
97. Marks A, Etteson D, Bjorn MJ, *et al.* Inhibition of human tumor growth by intraperitoneal immunotoxins in nude mice. *Cancer Res* 1990; **50**: 288–92.
98. Griffin T, Raso V. Monensin in lipid emulsion for the potentiation of ricin A chain immunotoxins. *Cancer Res* 1991; **51**: 4316–22.
99. Jähade E, Glüsenkamp K, Rajewsky MF. Nigericin enhances mafosfamide cytotoxicity at low extracellular pH. *Cancer Chemother Pharmacol* 1991; **27**: 440–4.
100. Grinstein S, Rotin D, Mason MJ. Na^+/H^+ exchange and growth factor-induced cytosolic pH changes. Role in cellular proliferation. Biochim Biophys Acta 1989; **988**: 73–7.
101 Niemann H. Post-translational glycosylation of corona virus glycoprotein E1: inhibition by monensin. *EMBO J* 1982; **1**: 1499–1504.

(Received 16 July 1992; accepted 30 July 1992)