Effects of New Triphenylethylene Platinum(II) Complexes on the Interaction with Phosphatidylcholine Liposomes

Guillaume GRENIER, Gervais BÉRUBÉ, and Claude GICQUAUD*

Département de Chimie Biologie, Université du Québec à Trois Rivières, CP 500, Trois Rivières, PQ, Canada G9A 5H7.
Received February 19, 1998; accepted June 1, 1998

In a previous work we synthesized a class of new antineoplastic drugs by coupling a cisplatin derivative to a triphenylethylene moiety similar to the antiestrogen, tamoxifen. These drugs differ in the number of hydroxy functions on the triphenylethylene rings and in the length of the linking arm.

To gain more insight into the cellular mechanism by which these new drugs act on cells, we studied, using differential scanning calorimetry, the effects of these compounds on the phase transition of membrane phospholipid (distearoyl phosphatidyl choline (DSPC)), and correlated these effects to drug cytotoxicity. The drugs without hydroxy function showed the highest cytotoxicity and induced little change on the thermogram of DSPC. Contrarily, the drugs bearing two or three hydroxy groups were less toxic, but induced important modifications of the thermogram.

We suggest that the drugs with no hydroxy group enter the membrane, with the triphenylethylene moiety localized deep within the hydrophobic core of the bilayer and do not affect the cooperativity region (C2—C8). In contrast, drugs which bear hydroxy groups on the triphenylethylene rings system perturb the phospholipid molecular arrangement; this may be due either to the additional steric hindrance of the hydroxy functions in the core of the bilayer, or to their hydrophilic effect on the polar head of the lipid.

***In vitro*, the cytotoxic effect of these drugs seems not to be related to their affinity for the estrogen receptor. We suggest that the addition of a triphenylethylene moiety to the platinum(II) complexes increases the hydrophobicity, and consequently the resulting drugs become more permeable to the membrane, particularly the non-hydroxylated triphenylethylene derivatives.**

Keys words cisplatin; tamoxifen; membrane fluidity; phospholipid phase transition; breast cancer; differential scanning calorimetry.

In our search for a chemotherapeutic agent with a better therapeutic index and selectivity for the treatment of breast cancer, we have designed dual action drugs made of a platinum coordinate complex linked to a triphenylethylene moiety.[1,2] The compounds synthesized differ from each other by the number of hydroxy groups on the triphenylethylene rings and the length of the arm which links the triphenylethylene moiety and the platinum(II) group (Fig. 1). The cisplatin portion of the molecule is known to exhibit a remarkable antineoplastic activity on a wide spectrum of tumors by acting on the guanine residue of the DNA molecule.[3,4] The triphenylethylene portion of the new derivatives is similar to the structure of tamoxifen which is an efficient drug used in the treatment of breast cancer.[5] Tamoxifen acts as an antiestrogen, antagonizing the binding of estradiol to the estrogen receptor (ER).[6] However, the multiple cellular effects of tamoxifen suggest that other mechanisms may be involved for its antiestrogenic action, including an effect on prostaglandin synthetase,[7] on Ca^{2+}-calmodulin-dependent enzymes,[8] on protein kinase C,[9] or an interaction with membrane phospholipids.[10,11]

In preceding papers,[1,2] we have shown that this new class of drugs is very active *in vitro* against ER+ and ER− human breast tumor cell lines: MCF-7 and MDA-MB-231. Surprisingly, all the compounds are more cytotoxic on ER− than on ER+ cell lines, showing that binding to the ER is not the unique site of action.

In this preliminary study, we have analysed the effect of these drugs on the thermotropic properties of lipid vesicles (liposomes) using differential scanning calorimetry (DSC), which has appeared very useful for the study of the interactions between drugs and membrane lipids.[12] We found that all these new drugs modified the transition temperature and cooperativity for the gel to liquid–crystalline transition of model membrane, and we correlated these changes with the cytotoxicity. The drugs without hydroxy function induced little change on the thermogram but have the highest cytotoxicity. In contrast, the drugs which possess hydroxy functions induced important modifications of the thermogram but are less toxic.

Experimental

Synthesis of Triphenylethylene Platinum(II) Complexes The synthesis of triphenylethylene platinum(II) complexes **1** shown in Fig. 1 have been described previously.[1,2]

Preparation of Liposomes Liposomes were prepared by the freeze-thawing technic.[13] Briefly, 10 mg of DSPC (distearoyl phosphatidyl choline) (Avanti Polar Lipids, Alabaster) and the quantity of triphenylethylene complexes necessary to get a molar ratio drug : DSPC = 1 : 9 were dissolved in a minimum amount of chloroform. The solvent was evaporated under vacuum in a rotary evaporator and the mixture resuspended in phosphate buffer, 5 mM and NaCl 150 mM, pH 7.4, by vortexing. The final concentration of lipids was 10 mg/ml. The lipid suspension was transferred to a cryovial, frozen in liquid nitrogen, and thawed in a waterbath at 70 °C; this freeze-thaw cycle was repeated 3 times. As we have seen by electron microscopy, these preparations contain mainly bilamellar liposomes 0.1—0.5 μm in diameter (data not shown).

DSC Prior to the scan, each sample was degassed for 5 min. under partial vacuum. DSC was performed with a HART 4207 system equipped with three stainless steel ampoules. Each ampoule was filled with 900 mg of liposome suspension. The scan rate was 60 °K/h. The baseline was obtained using the buffer alone and was subtracted from the samples. Calorimetric enthalpy was calculated using a program furnished with the instrument. The average size of the transition cooperative unit was calculated from the measured calorimetric enthalpy, ΔH_{cal}, using the following equation[14] $CU = (6.9/\Delta H_{cal})(T 2_m/\Delta T_{1/2})$.

Results

Figure 2 presents the calorimetric heating curves of DSPC

* To whom correspondence should be addressed.

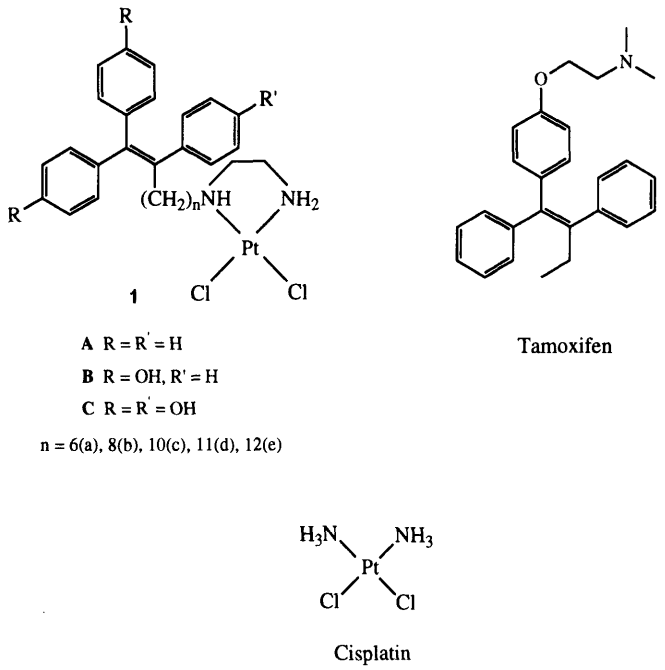

Fig. 1. Structure of Tamoxifen, Cisplatin and the Triphenylethylene Platinum(II) Complexes **1**

Group A, R=R'=H; Group B, R=OH, R'=H; Group C, R=R'=OH. Number of carbons of the linking arm: (a) $n=6$; (b) $n=8$; (c) $n=10$; (d) $n=11$; (e) $n=12$.

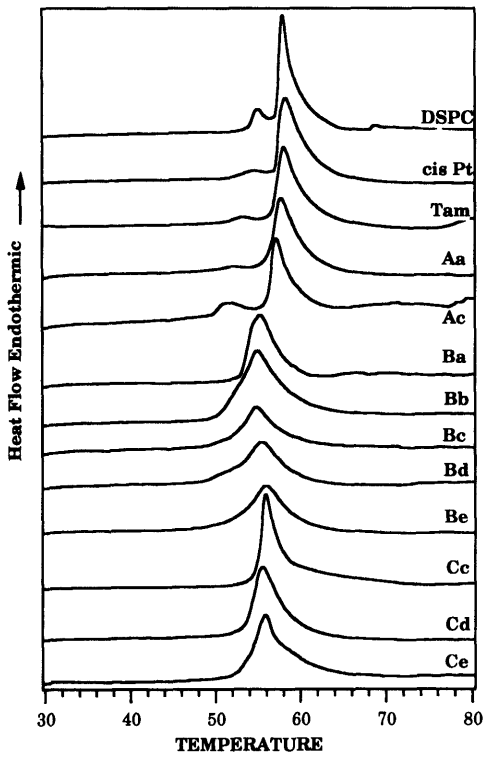

Fig. 2. DSC Thermograms for the Thermotropic Transition of Liposomes Composed with DSPC and Triphenylethylene Platinum(II) Complexes at a Molar Ratio 9 : 1

liposomes in the absence and the presence of the triphenylethylene platinum(II) complexes that we have synthesized. The thermogram of DSPC alone has two peaks. The first one at 55 °C is the pretransition and corresponds to a change of the bilayer from an Lβ' organization to a Pβ'phase, and the second peak at 57.9 °C is the main transition and corresponds to the melting of the lipid molecules to a liquid crystal structure or Lα. This thermogram of DSPC corresponds to those previously published.[15]

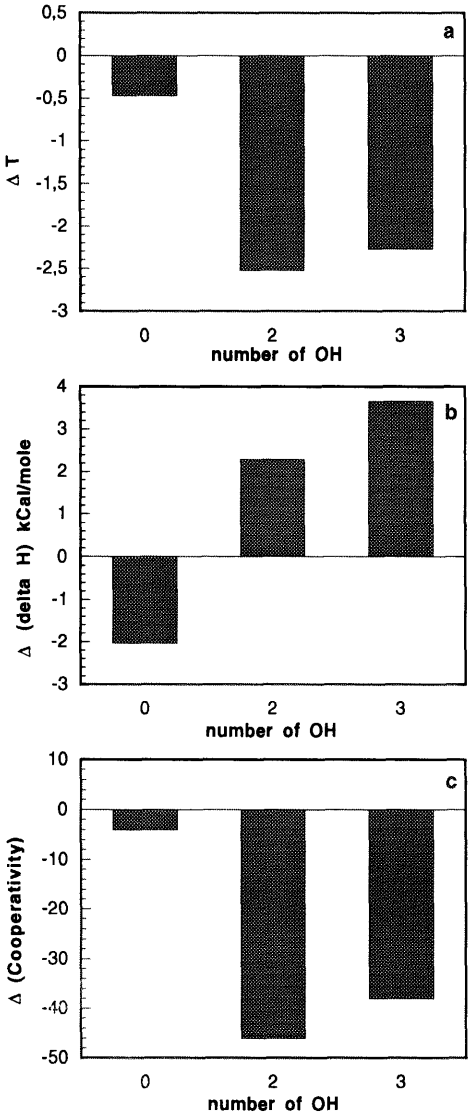

Fig. 3. Influence of the Number of Hydroxy Functions on Triphenylethylene Rings

On: (a), The shift of the transition temperature (ΔT); (b), variation of the calorimetric enthalpy (ΔH); and (c), variation of the cooperativity. The results are an average of several thermograms. The liposomes were made with DSPC and triphenylethylene platinum(II) complexes at a molar ratio 9 : 1; the reference was DSPC alone.

As we can see in Fig. 2, all the drugs interact with DSPC liposomes and the effect depends on their structure. As judged from the curves, the substances can be divided into three groups. The first group induces only a small change in the aspect of the thermograms. The drugs of this group have the peculiarity of being devoid of hydroxy function (group A). The second group induces an important shift in the transition temperature and a broadening of the peaks. All the substances of this group possess two hydroxy functions (group B). The third group, which includes compounds bearing three hydroxy functions shows an intermediate effect (group C). Tamoxifen and cisplatin which are the reference substances produced only slight effects on the thermogram of DSPC as did the drugs of group A.

Figure 3 compiles the results obtained from several thermograms and helps us to visualize the effects of the number

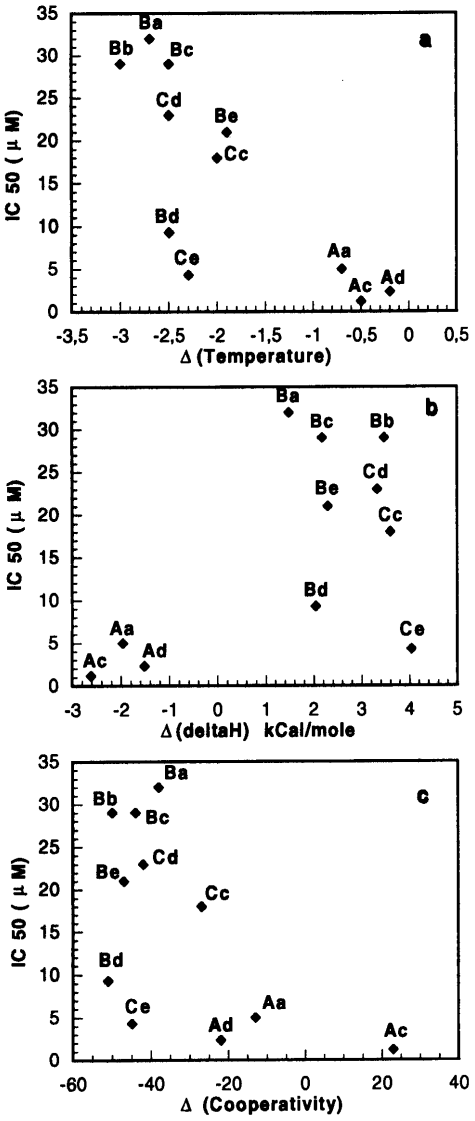

Fig. 4. Correlation between the IC_{50} of the Free Drug on ER− Human Breast Tumor Cell Line MDA-MB-231

With: (a), shift of the transition temperature (ΔT); (b), variation of the calorimetric enthalpy (ΔH); and (c), variation of the cooperativity. Group A, R=R′=H; Group B, R=OH, R′=H; Group C, R=R′=OH. Number of carbons of the linking arm: (a) n=6; (b) n=8; (c) n=10; (d) n=11; (e) n=12.

of hydroxy functions and the number of carbons in the linking arm. Figure 3a shows that the drugs without hydroxy function reduced the transition temperature by only 0.5 °K while those which have two or three hydroxy functions produced an important shift of 2.5 °K. As can be seen in Fig. 3b, the drugs without hydroxy function decreased the calorimetric enthalpy while those bearing hydroxy functions increased it. Figure 3c illustrates that the drugs with two hydroxy functions greatly decreased the cooperativity of the phase transition while those with three hydroxy functions were slightly less effective. Interestingly, the drugs without hydroxy function have no effect on the cooperativity. Therefore, the presence of hydroxy group on the molecule greatly influences its interaction with the lipids. The number of carbons of the linking arm, however, has no significant effect on the thermograms (data not shown).

Figures 4a—c correlate the inhibitory concentration (IC_{50}) of the different platinum(II) complexes on the MDA-MB-231 (ER−) cell line with several parameters obtained from the thermograms. The IC_{50} were determined earlier.[1,2] There is a correlation between the inhibitory concentration of the drugs and their effects on the transition temperature of the lipid (r=0.77); the compounds showing the highest effect on the transition temperature have the highest IC_{50} and, consequently, the lowest toxicity. The correlation between the inhibitory concentration and the effect on calorimetric enthalpy is less clear (r=0.58). However, in Fig. 4b, one can see two sets of points, those in the lower left corner correspond to the drugs of group A that possess no hydroxy function: they are the most toxic and decrease the calorimetric enthalpy, while the drugs in the upper right corner are less toxic and increase the calorimetric enthalpy, belonging to groups B and C bearing two or three hydroxy functions. The correlation between the IC_{50} and the cooperativity is not evident (r=0.57), and one cannot see any distinct cluster of points (Fig. 4c). The same observations were made with the MCF-7 (ER+) cell line (results not shown).

We can thus conclude that the addition of hydroxy functions to the triphenylethylene moiety of the platinum complexes produces an important effect on the thermogram, and this is accompanied by a diminution of the toxicity of the drug.

Discussion

DSC is now widely used to study the influence of drugs on membrane lipids. Several hundred compounds have been studied and shown to induce modifications of the phase transition profile depending on their localisation in the lipid bilayer. The pretransition which is ascribed to the transition from a Lβ' to a Pβ' structure is very sensitive to the presence of molecules able to exert an action on the polar head of the lipid. Therefore, it is not very specific since many molecules which do not enter into the lipid bilayer can still influence the polar head from the outside.[16] However, the main transition corresponds to the change in the organisation of the lipid from a gel phase, or Lβ where the acyl chains are in all *trans* conformation to a liquid crystalline, or Lα where the acyl chains are fluid because they display an increased *trans*–gauche isomerisation. The total enthalpy change associated with the main lipid chain melting is related to molecular packing of the acyl chains.[17] Thus, any drug entering the bilayer will modify the interacting forces between the acyl chains of the phospholipids and, consequently, the aspect of the thermogram. In most cases, insertion of a drug into the lipid bilayer will affect the thermogram. It was demonstrated that a broadening of the peak profile with a decrease or an increase in the melting temperature suggests location of the drug in the cooperative region corresponding to the first eight carbons of the acyl chain (C2—C8 region).[18] It was also observed that a shift of the peak to lower temperature without broadening is the result of an interaction at the C10—C16 level.[12]

The reference substance, tamoxifen, which has no hydroxy function, only slightly affects the thermogram of DSPC. Custódio *et al.*[11] suggested that tamoxifen molecules are located in the hydrophobic interior region of the ordered bilayer and do not affect the cooperativity region (C2—C8). The new derivatives of the group A that we made do not modify significantly the cooperativity. Since they contain the triphenylethylene ring system found on tamoxifen, we sug-

gest that they also are deeply inserted in the hydrophobic core of the bilayer, below the C2—C8 region.

Contrarily, the molecules of groups B and C, which bear two and three hydroxy functions on the phenyl rings, greatly broaden and shift the peak of the thermogram to lower temperature. This can be interpreted in two ways: the hydroxy functions certainly lead to additional steric hindrance, creating voids between the acyl chains which are compensated by an increase in the ratio of gauche to *trans* conformers and that destabilize the gel phase or, the hydroxy functions may have an hydrophilic effect on the polar head group of the lipids and the drug may partially extend its distribution into the outer hydrophobic region of the bilayer producing a pronounced broadening of the transition profile.

When we synthesized these new compounds, we were expecting that their mechanism of action would be solely through the binding of the triphenylethylene moiety to the ER followed by the cytotoxic action of the platinum(II) portion of the molecule. Consequently, we thought that this type of compound would accumulate more efficiently in the ER+ breast cancer cells. *In vitro* this was not the case, since we established that the ER− cells are more sensitive than the ER+, and the drugs of group A which are the more toxic have no affinity for the ER while those of groups B and C which bind to the ER are less toxic.[1,2]

The major target of these drugs into the cell nucleus is most likely DNA. Membrane interaction, membrane passage, drug activation, and DNA-adduct formation will, together with other factors, determine cytotoxicity. A change in the structure of cisplatinum analogue may affect any of these factors. However, the results presented here suggest that the addition of the triphenylethylene rings to the platinum(II) complexes will increase the hydrophobicity, and consequently the resulting drug becomes more permeable to the membrane, particularly the non-hydroxylated triphenylethylene derivatives.

Acknowledgements The authors acknowledge Serge Groleau and Yuehua He for technical help and Daphne Efford for revision of the manuscript. This work was supported by grants from the Fonds pour la Formation et l'Aide à la Recherche (FCAR Québec) and le Conseil en Sciences Naturelles et Génie Canada (CRSNG).

References

1) He Y., Groleau S., Caron M., Thérien H.-M., Bérubé G., *Bioorg. Med. Chem. Lett.*, **5**, 2217—2222 (1995).
2) Bérubé G., He Y., Groleau S., Séné A., Thérien H.-M., Caron M., *Inorganica Chimica Acta*, **262**, 139—145 (1997).
3) Hydes P. C., Russell M. J. H., *Cancer and Metastasis Rev.*, **7**, 67—89 (1988).
4) Lemaire D., Fouchet M.-H., Kozelka J., *J. Inorg. Biochem.*, **53**, 261—271 (1994).
5) Fahey S. M. L., Jordan V. C., Fritz N. F., Robinson S. P., Waters D., Tormey D. C., "Long-term Tamoxifen Treatment for Breast Cancer," ed. by Jordan V., University of Wisconsin, Madison, 1994, pp. 27—56.
6) Lazier C. B., Bapat B. V., *J. Steroid Biochem. Mol. Biol.*, **31**, 665—669 (1988).
7) Gilbert J., Miquel J. F., Précigoux G., Hospital M., Raynaud J. P., Michel F., Paulet A., *J. Med. Chem.*, **26**, 683—699 (1983).
8) Lam H.-Y., *Biochem. Biophys. Res. Commun.*, **118**, 27—32 (1984).
9) O'Brian C. A., Liskam R. M., Solomon D. H., Weinstein I. B., *J. Nat. Cancer Inst.*, **76**, 1243—1246 (1986).
10) Custódio J. B. A., Almeida L. M., Madeira V. M. C, *Biochim. Biophys. Acta*, **1153**, 308—314 (1993).
11) Custódio J. B. A., Almeida L. M., Madeira V. M. C., *Biochim. Biophys. Acta*, **1150**, 123—129 (1993).
12) Bach D., "Biomembrane Structure and Function: Topics in Molecular and Structural Biology," Vol. 4, Verlag, ed. by Weinkeim V., Chapman D., 1984, pp. 1—41.
13) Bally M., Hope M., Mayer L., Madden T., Cullis P., "Liposomes as Drug Carriers," ed. by Gregoriadis D., Jhon Willey & Sons, Toronto, 1988, pp. 841—853.
14) Hinz H. J., Sturtevant J. M., *J. Biol. Chem.*, **247**, 6071—6075 (1972).
15) Blume A., *Thermochimica Acta*, **193**, 299—347 (1991).
16) Cater B. A., Chapman D., Hawes S., Saville J., *Biochim. Biophys. Acta*, **363**, 54—69 (1974).
17) Ramaswami V., Haaseth R. C., Matsunaga T. O., Hruby V. J., O'Brien D. F., *Biochim. Biophys. Acta*, **1109**, 195—203 (1992).
18) Jain M. K., Wu N. M., *J. Membrane Biol.*, **34**, 157—201 (1977).