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News and Views

# Biodistribution of a Zn(II) phthalocyanine to plasma lipoproteins

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Among the second-generation photosensitizers which have recently been synthesized is a substituted Zn phthalocyanine (ZnBPc), initially described in Ref. [1]. Since this sensitizer is poorly soluble in aqueous media, a formulation procedure is required for biologic studies. Soncin et al. have reported on the marked affinity of ZnBPc for low density lipoproteins (LDLs) in the tumor-bearing mouse; drug solubilization with dipalmitoylphosphatidyl choline liposomes resulted in the binding of more than 75% of the circulating drug to LDL in the mouse in vivo, while the use of Cremophor EL for formulation resulted in an almost quantitative binding of ZnBPc to mouse LDL [2]. In the latter case, ZnBPc accumulation by mouse tumor tissues in vivo was greatly enhanced.

These results are in agreement with the proposal [3] that binding of photosensitizing agents to LDL will enhance the selectivity of PDT, since neoplastic tissues generally express elevated levels of LDL receptors [3]. In this report, we examine the affinity of ZnBPc for human lipoproteins in vitro as a function of the drug formulation procedure. Additional studies were carried out to assess ZnBPc aggregation in the different media, so as to provide a possible explanation for the observed results.

Human pooled normal plasma (250 $\mu$l) was incubated with 0.3 $\mu$l of 10 mM ZnBPc for 30 min at 37 °C. The drug was solubilized in 20% Cremophor EL (CRM), 2% Tween 80 (TW80) or 100% dimethylformamide (DMF). Final concentrations of CRM (0.024 mg $ml^{-1}$) or TW80 (0.002 mg $ml^{-1}$) were below the threshold required for lipoprotein alterations [4]. Mesoporphyrin (MP) was also added to the plasma samples at a concentration of 1 $\mu$M, to facilitate identification of protein and lipoprotein fractions [5]. LDL, high density lipoprotein (HDL) and heavy proteins (mainly albumin) were separated by density gradient ultracentrifugation [4,5]. The concentration of ZnBPc in the different fractions was assessed by fluorescence, using 650 nm excitation, with fluorescence determined at 745 nm. MP fluorescence was detected using 400 nm excitation and 630 nm fluorescence. The fluorescence properties of ZnBPc and MP are such that there is no signal overlap between the two compounds.

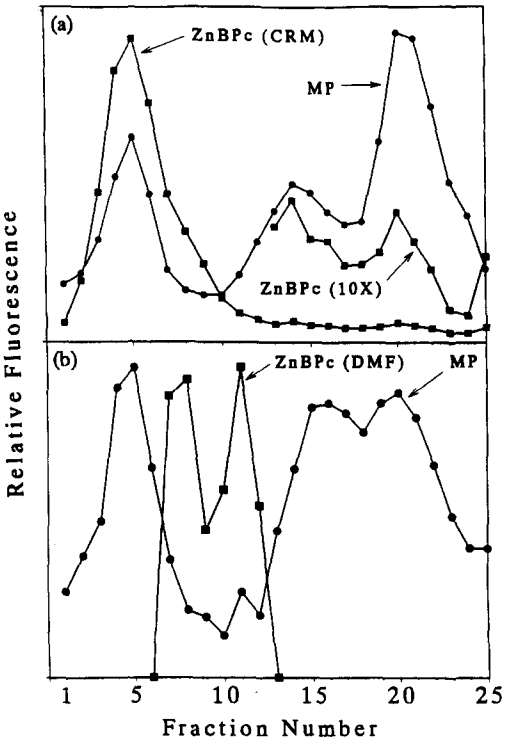


Fig. 1. Distribution of MP and ZnBPc to human plasma fractions in vitro as assessed by density gradient ultracentrifugation. (a) ZnBPc in 20% CRM (■) and aqueous MP (●). Also shown, ZnBPc at 10X sensitivity. (b) ZnBPc in DMF (■) and MP (●). In these studies, LDL is centered at fractions 4–5, HDL at 13–15 and albumin at 20–21.

Using MP as the tracer, we found LDL centered in fraction 5, HDL at fractions 14–15 and albumin at 20–21. When DMF was used to solubilize ZnBPc, density gradient ultracentrifugation indicated localization of the agent to two fractions of buoyant density between HDL and LDL (Fig. 1(b)). When either CRM or TW80 was used for formulation, more than 90% of the ZnBPc was found in the LDL fraction (Fig. 1(a)). The sensitivity of the fluorescence detector was increased by a factor of 10 to show the minor degree of binding of ZnBPc to HDL and "heavy" proteins.

In an attempt to discover the rationale for this behavior, we examined the 726 nm absorbance of solutions of ZnBPc


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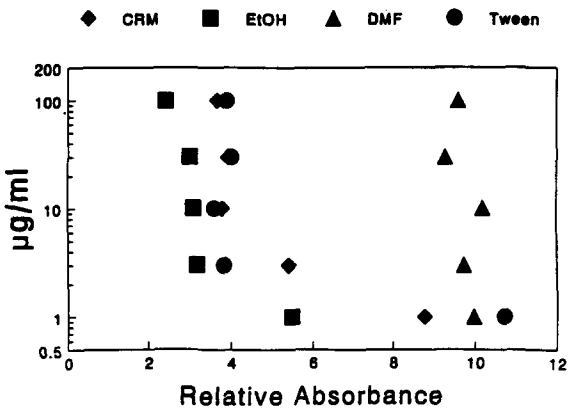


Fig. 2. Relative absorbance of solutions of ZnBPc in 20% CRM, ethanol, DMF or 20% Tween 80. In each case, the sensitivity of the instrument was increased proportionally to the dilution of the ZnBPc solution.

in ethanol, DMF, 20% TW80 and 20% CRM (Fig. 2). Absorbance was proportional to concentration over a broad range (1–100 $\mu$M) in DMF, but the fall in relative absorbance at concentrations above 3 $\mu$M in CRM or TW80 suggests the formation of aggregates at higher ZnBPc concentrations. Some aggregation in ethanol was also suggested at ZnBPc concentrations above 3 $\mu$M. Rihter et al. [1] observed no aggregate formation of ZnBPc in benzene, but data shown in Fig. 2 suggest aggregate formation in CRM and TW80 and raise the possibility that these aggregates may be responsible for the affinity of ZnBPc for LDL. Alternatively, there may be an alteration in LDL by TW80 or CRM too subtle to be detected by ultracentrifugation or electrophoresis which is responsible for the binding of the dye to LDL.

The role of Cremophor EL as a factor in PDT efficacy remains to be explored in more detail. What initially appeared to be a preferential partition of sensitizers into LDL by Cremophor [6] later turned out to derive from CRM-induced alterations in plasma lipoproteins [4,7]. The remarkable affinity of ZnBPc for mouse LDL [2] suggests that this agent may also be an effective PDT sensitizer in man, where the relative proportion of LDL is substantially greater than in the mouse [8].

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## References

[1] B.D. Rihter, M.D. Bohorquez, M.A.J. Rodgers and M.E. Kenney, Two new sterically hindered phthalocyanines: synthetic and photodynamic aspects, *Photochem. Photobiol., 55* (1992) 677–680.

[2] M. Soncin, L. Polo, G. Jori, B.D. Rihter, M.E. Kenney and M.A.J. Rodgers, Unusually high affinity of Zn(II)-tetradibenzobarrelenooctabutoxyphthalocyanine for low-density lipoproteins in a tumor-bearing mouse, *Photochem. Photobiol., 61* (1995) 310–312.

[3] J.C. Mazière, P. Morlière and R. Santus, The role of the low density lipoprotein receptor pathway in the delivery of lipophilic photosensitizers in the photodynamic therapy of tumours, *J. Photochem. Photobiol. B: Biol., 8* (1991) 351–360.

[4] K. Woodburn and D. Kessel, The alteration of plasma lipoproteins by cremophor EL, *J. Photochem. Photobiol. B: Biol., 22* (1994) 197–201.

[5] E. Sykes, M. Meany, V. Schulz and D. Kessel, Separation of plasma lipoproteins on a table-top ultracentrifuge, *Clin. Chim. Acta, 205* (1992) 137–144.

[6] G.M. Garbo, The use of liposomes, emulsions or inclusion complexes may potentiate in vivo effects of SnET2, *Proc. Soc. Photo-Opt. Instrum. Eng., 1203* (1990) 118–125.

[7] M. Kongshaug, L.S. Cheng, J. Moan and C. Rimington, Interaction of cremophor EL with human plasma, *Int. J. Biochem., 23* (1991) 473–478.

[8] M.J. Chapman, Comparative analysis of mammalian plasma lipoproteins, *Methods Enzymol., 128* (1986) 70–143.