Impact of transport proteins on pharmacokinetics and photodynamic efficacy of chlorophyll derivatives

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Pharmacokinetics of drugs used in photodynamic therapy is of crucial importance for both the efficacy of therapy and patient safety. In the present study, two factors critical for the distribution of chlorophyll-derived photosensitizers (PSs) in the body, i.e. their transport *via* the breast cancer resistance protein (BCRP, ABCG2) and binding to serum albumin, were analyzed. The investigated compounds were chlorophyllide a and zinc-pheophorbide a, differing only in central metal ion substituting the tetrapyrrole ring. Interactions of these drugs with BCRP and albumin were studied in parallel with the analysis of their impact on the photodynamic efficacy of the PSs.

Transport studies were performed using the MCF-7 cell line (breast cancer) with basal and transiently up-regulated expression of BCRP. In parallel, photodynamic effect induced by the PSs remaining in the cells after a defined period of transport was determined. It was shown that both magnesium- and zinc-substituted derivatives of pheophorbide a are substrates of the transporter, but their transport rate is lower than that of pheophorbide a, which is a previously confirmed substrate of BCRP. Additionally, lower transport rates were observed for chlorophyllide than for zinc pheophorbide. This is most likely due to differences in their affinity to the extracellular loop ECL3 of the transporter. At the same time, it was observed that the transport rate of both derivatives, like that of pheophorbide a, is significantly reduced in the absence of serum/albumin in the extracellular environment, similarly as for other compounds of similar structure. The rate of transport increases with increasing concentration of albumin, reaching a plateau at about 250 μM of the protein. The accelerated transport may be due to the binding of a PS to albumin, which prevents the complex from re-entering the cell. However, this might not be the only mechanism of this phenomenon, since no correlation was observed between the rate of PS transport and its affinity for albumin. It was observed that metal substituted derivatives bind to albumin much stronger than pheophorbide a. Additionally, this binding is stronger for the zinc-substituted derivative than for the magnesium one. Presumably, this difference, rather than the difference in the rate of transport by BCRP as previously speculated, is responsible for the prolonged retention time in the body of the zinc derivative compared to chlorophyllide observed in previous *in vivo* studies.

To thoroughly characterize the binding of the PSs to human serum albumin, spectroscopic and molecular docking studies were performed. Binding constants were determined by the measurement of the PSs fluorescence enhancement with increasing concentration of albumin. Probable binding sites, in turn, were identified by fluorescence quenching studies and molecular

docking. These studies showed that chlorophyll derivatives bind most strongly at the heme binding site located in subdomain IB, and weaker at the major albumin binding sites, i.e. Sudlow1 and Sudlow2, located in subdomains IIA and IIIA, respectively. This binding is predominantly based on hydrophobic interactions and, to a lesser extent, hydrogen bonding. The determined binding constants were lower for chlorophyllide than for zinc pheophorbide. Moreover, the number of identified amino acid residues involved in the binding to albumin was lower for the former than for the latter. This suggests that the differences in their pharmacokinetics observed in animals are also likely to occur in humans.

Because of relatively strong binding of zinc pheophorbide a to albumin, which hinders its uptake by cancer cells, and on the other hand high photodynamic potential of this derivative, it was reasonable to investigate whether the complex of albumin with zinc pheophorbide enters vascular endothelial cells, which express the albumin receptor, gp60, involved in the uptake of this protein by endocytosis. The experiments performed using the HUVEC model cell line (endothelial cells isolated from the umbilical vein) confirmed that the complex is incorporated into the cells. Moreover, complexation of the PS with albumin increases its lysosomal accumulation, thus enhancing the induced photodynamic effect. It was also shown that HUVECs are much more susceptible to photodynamic treatment with zinc pheophorbide than MCF-7 cancer cells. The IC50 value for the HUVEC cell line at a light dose of 2 J/cm^2 and a 3-hour accumulation time was about 20 nM, while for the MCF-7 line this value is approx. 25 times higher. It was also shown that the type of cell death induced by photodynamic treatment depends on the level of zinc pheophorbide a accumulated in the cells. At low levels of the PS, cells undergo apoptosis and at high levels, necrosis. Since high concentrations of albumin significantly reduce cellular accumulation of the PS, they will also direct cells into the apoptosis pathway, which may be a beneficial effect, due to the reduction of inflammation.