Uptake of photofrin II, a photosensitizer used in photodynamic therapy, by tumour cells *in vitro*

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Photosensitizing dyes are used in fluorescence diagnostics and photodynamic therapy (PDT). These usually hematoporphyrin derivatives (HpD) accumulate preferentially within neoplastic tissues. HpD is a mixture of ether and ester linked porphyrins. Its partially purified form is known under the commercial name of photofrin II (PII).

PII emission spectra were studied in a hydrophilic (PBS) and a lipophilic (PC liposomes) environment. Red shift was observed in their emission maxima from 615 nm in buffer solution to 635 nm in lipid. Identical red shift was obtained when the intracellular fluorescence of two cancer cell lines, MCF 7 and Jurkat, incubated with PII was investigated. Thus, intramembrane localization of PII may be suggested. As determined from the total fluorescence intensity, the uptake of PII was only slightly higher for Jurkat than for MCF 7 cells. Nevertheless the kinetics of the uptake was found to be different for both cell lines.

Photodynamic therapy (PDT) is an emerging treatment for a variety of conditions, but particularly for cancer and for wet age-related macular degeneration. PDT is a cancer treatment involving incorporation of photosensitizing molecules into malignant tumours and their activation with visible light. In general porphyrin-type dyes have been employed as photosensitiers in cancer phototherapy because of their properties: high singlet oxygen quantum yields, apparent lack of dark toxicity, and absorption in the red region

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(Ochsner, 1997). HpD is an unstable mixture of porphyrin monomers and oligomers joined by ether and ester linkages. The antitumour activity basically resides in the covalently-linked oligomeric fraction, and therefore in the commercial preparations the less active monomeric fraction is removed using HPLC or gel permeation chromatography (Sternberg *et al.* 1998). This more purified version called PII, was the first photosensitizer employed in PDT. PII was authorized for use against certain types of bladder tumour in Canada, U.S.A., Japan, and some European countries.

Upon photoactivation, PII may produce high quantum yield of singlet oxygen and/or superoxide anions, and even semiquinone radicals (Foster *et al.*, 1991; Kriska *et al.*, 1995; Fernandez *et al.*, 1997). Once generated, each one of these biochemical species may cause cell injury (Furuse *et al.*, 1993). Tumours selectively take up PII, but the process of photosensitizer localisation, which favours tumour accumulation, is not really understood. This effect is partly related to the high lipophilicity of PII (Ricchelli, 1995). In *in vivo* treatments, after intravenous administration of hydrophobic sensitizers, the compounds are, in general, strongly bound to lipoproteins, high density lipoproteins (HDLs) and low density lipoproteins (LDLs), distributed with the blood system and transported to the malignant tissue with distinct selectivity (Gèze *et al.*, 1994; Byrne *et al.*, 1990). It is well accepted today that the tumour selectivity increases with the lipophilic character of the sensitizing agent. An examination of the components of PII reveals that the therapeutically active lipophilic fraction consists of a heterogenous and poorly reproducible mixture of different porphyrins with various degrees of oligomerization (from monomers to hexamers) and chemical structures (ether and ester linkages between porphyrin units) (Byrne *et al.*, 1990). In solution these oligomers assume a compact structure with the individual porphyrin rings stacked over one an-

other, and the concurrent increase in hydrophobicity leads to enhanced intracellular accumulation (Ochsner, 1997). Differences in the clearance rates of sensitizers between tissues enable one to establish the most appropriate timing of irradiation of neoplastic tissue. The preferred accumulation of lipophilic sensitizers, such as PII, within tumour tissues is in reasonable harmony with the observation that neoplastic cells express a particularly large number of membrane receptors for LDLs (Korbelik, 1993; Biade *et al.*, 1992). *In vitro* and *in vivo* studies have clearly demonstrated the role of the LDL receptor pathway in the delivery of lipophilic sensitizers to tumour cells. Following receptor-mediated endocytosis, the sensitizer molecules preferentially accumulate in the lipophilic compartments of tumour cells, including the plasma, mitochondrial, endoplasmic reticulum, nuclear and lysosomal membranes. The purpose of this study was to investigate the kinetics of uptake of two cancer cell lines MCF 7 and Jurkat.

MATERIALS AND METHODS

Chemicals. PII, MEM, L-glutamine and trypsin were obtained from Sigma, fetal bovine serum (FBS) was purchased from Bio-Whittaker, phosphate-buffered saline (PBS) was obtained from IITD (Wrocław, Poland).

L-α-Phosphatidylcholine (EYPC) from egg yolk was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, U.S.A.)

Liposome preparation. Unilamellar EYPC liposomes were obtain by sonication of 1mM phospholipid suspension in PBS using a UP200s sonicator (Dr. Hilsder GmbH, Berlin, Germany)

Culture cells. The cell model used consisted of breast adenocarcinoma MCF 7 cells and leukaemic T cell lymphoblast Jurkat cells. The cells were allowed to grow in MEM medium supplemented with 10% FBS and glutamine

(2 mM) in a humidified CO_2 atmosphere at 37°C. MCF 7 cells were adherent to the culture support and detached by trypsinization (trypsin 0.025%, EDTA 0.02%).

PII incubation for fluorescence measurement. PII was dissolved according to the manufacturer's recommendations in PBS to obtain a 0.6 mg/ml stock solution. Cells were incubated for times varying from 2 to 24 h in the described conditions in the presence of PII (final concentration 30 μg/ml) in the dark. MCF 7 cells were trypsinized and both cell lines were washed three times with BPS, counted and diluted to the same cell concentration (10^5 cells/ml).

Fluorescence imaging (spectra). Fluorescence intensity was measured at the excitation wavelength of 410 nm by using an LB 50B spectrofluorimeter (Perkin-Elmer, Beaconsfield, U.K.). The fluorescence spectra were recorded in the 450–700 nm range.

RESULTS AND DISCUSSION

Emission spectra of photofrin II

In the present study PII emission spectra were obtained in a hydrophilic (PBS) and a lipophilic (PC liposomes) environment (final concentration of PII 3 μg/ml). It can be seen that upon going from the aqueous solution to the more hydrophobic liposomal environment the drug undergoes a red shift in its emission maxima from 615 nm to 635 nm. A considerable increase of fluorescence intensity was noticed for the same concentration of PII as the medium hydrophobicity increased (Fig. 1).

An identical shift also occurs in the measurement of emission of PII taken up by MCF 7 and Jurkat cells. This suggests intramembrane localization of PII (Fig. 2).

This red shift indicates that the photosensitizer incorporated into cell membranes and this led to the change in its spectral properties. The subcellular dye localisation is the key factor in PDT because it determines the

site of the first cell damage after irradiation. The emission fluorescence spectrum of PII was investigated by Candide *et al.* (1986). They observed that addition of LDL to the solution increased the intensity and induced a
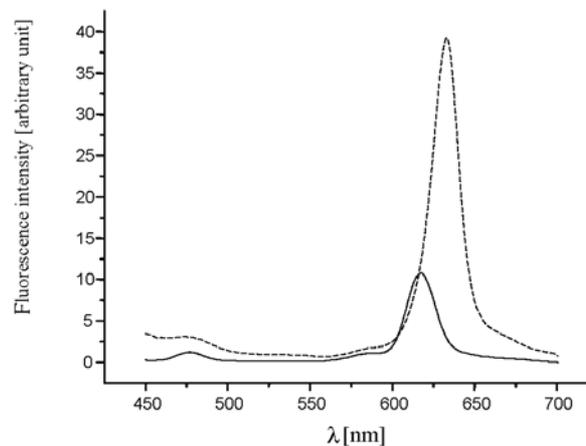

Figure 1. Fluorescence spectra of PII (3 μg/ml) in PBS buffer (——) and in EYPC liposomes (– – –) at excitation wavelength of 410 nm.
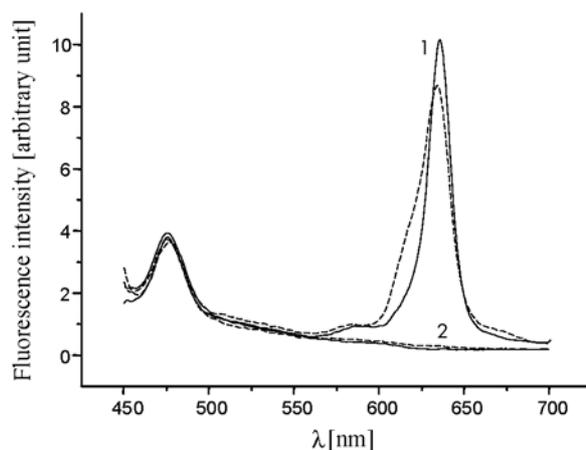

Figure 2. Fluorescence emission spectra at excitation wavelength of 410 nm from MCF 7 (– – –) and Jurkat (——) cells incubated with PII (30 μg/ml) for 5 h (1) and without photosensitizer (2).

red shift of the emission maximum from 613 nm to 627 nm.

Comparative photofrin II uptake into MCF 7 and Jurkat cells

PII uptake was determined by studying fluorescence emission of the drug after various in-

cubation periods using fluorescence spectroscopy. The fluorescence emission from the cells incubated with PII (30 μg/ml) appeared as a band with a peak at 635 nm (Fig. 2). The amount of PII uptake, as determined from the total fluorescence intensity, was found for Jurkat cells to be slightly higher than for MCF 7 cells but the kinetics of the accumulation appeared quite different. Differences in the rate of dye uptake and efflux were observed. For Jurkat cells absorption of the dye reached the maximum within 3 h and for MCF 7 cells the fluorescence rose more slowly and was maximal after 24 h (Fig. 3).

Protoporphyrin and hematoporphyrin have been demonstrated to bind to albumin and lipoproteins, mainly LDLs and HDLs. LDL uptake by cells is achieved by receptor-mediated endocytosis which results in LDL-component

ference of the photosensitizer with LDL recognition by the specific receptors. The cells were induced in a medium containing lipoprotein-deficient serum to achieve overexpression of LDL receptors. The hematoporphyrin—LDL complex was accumulated by human HT1080 fibroblasts mainly through high affinity LDL-receptors. The uptake of the LDL-delivered hematoporphyrin was about 4-fold higher in HT1080 cells stimulated for maximal expression of LDL receptors as compared with non-stimulated cells but the same complexes are taken up by stimulated and non-stimulated 4R rat fibroblasts with a similar efficiency (Polo *et al.*, 2002).

Therefore not only induction of LDL-receptors changes the uptake of the photosensitizer by the cell but also the differences in cell morphology influence the rate of PII up-
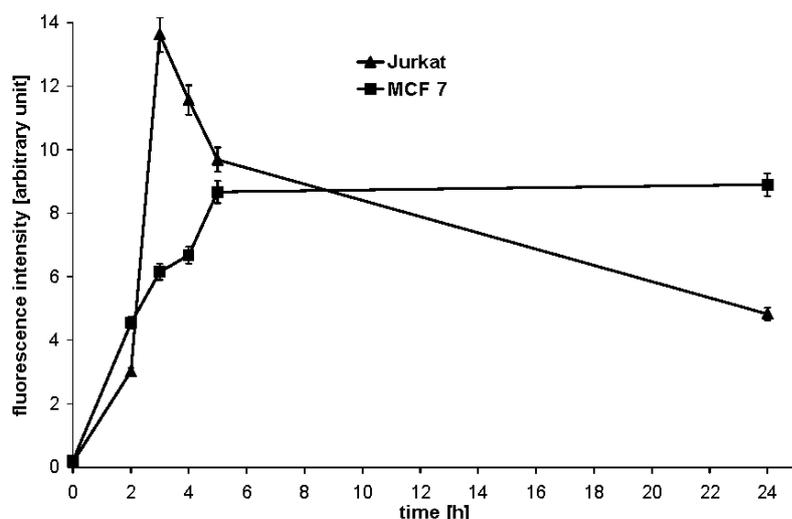

Figure 3. PII uptake (incubation concentration, 30 μg/ml) by MCF 7 and Jurkat cells.

Cells were incubated for times varying from 2 to 24 h in the presence of PII (incubation concentration, 30 μg/ml) in the dark.

delivery to the cytosomal compartment. Our results indicate that MCF 7 and Jurkat cells may differ in membrane composition or LDL receptor presentation which in consequence leads to different distribution of the dyes in the cells.

Photosensitizer uptake has been investigated in other studies. Reduction of the LDL-receptor number by preincubation of fibroblasts in a medium supplemented with lipoproteins resulted in a decrease of P II delivery (Candide *et al.*, 1986). Polo *et al.* (2002) compared the accumulation of PII by human and rat transformed fibroblasts and the inter-

take. Our investigations confirm the distinction in PII uptake between different cells. The work of Fickweiler *et al.* (1999) revealed a difference in the cellular uptake of another photosensitizer ATMPn (9-acetoxy-2,7,12,17-tetrakis-(β-methoxyethyl)-porphycene) beetwen some cell lines. This indicates that a given cell type has its own ratio of photosensitizer uptake and clearance. This suggests that the susceptibility of different cancer lines to PDT depends on various factors, among other membrane composition.

A very important issue resulting from these investigations is whether one can predict the efficiency of PDT and establish the optimal conditions of PDT for tumours of a given cell morphology. Further studies are needed to elucidate this problem.

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