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pH dependent uptake of porphyrin-type photosensitizers by solid tumor cells in vitro is not induced by modification of transmembrane potential

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Abstract

The uptake of HpIX, $TPPS_{2a}$ and mTHPC by WiDr, THX cells and skin fibroblasts at pH 7.4 and 6.8 was compared. In the absence of serum, the uptake of HpIX was higher at lower pH. The difference was significant in WiDr cells ($P<0.01$) and skin fibroblasts ($P<0.05$). $TPPS_{2a}$ nor mTHPC showed any pH dependent uptake. Lowering the extracellular pH resulted in a significant depolarization (3–8 mV) of the cells. Application of tetraethylammonium chloride did not affect the cellular uptake of any of the photosensitizers. We conclude that the pH dependent uptake of photosensitizers is not mainly related to altered transmembrane potential.

Keywords: Photodynamic therapy; pH-dependence; Transmembrane potential; Cellular uptake

1. Introduction

Tissue specific treatment effects are important in cancer therapy to avoid damage of normal tissue. In photodynamic therapy (PDT), the selectivity is achieved mostly by confining the illumination to the tumor tissue. In addition, indications of selective distribution of photosensitizers in tumor tissues have been reported [1–4]. Since diffuse, multifocal malignant lesions and disseminated tumors are important indications for PDT, it is desirable to increase the tissue selective distribution of photosensitizing drugs. However, the mechanisms that induce such selectivity and the factors that modify it have to be clearly understood to further improve tissue selectivity. Among the factors that have been considered until now, significantly lower pH in the tumors as

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compared to normal tissues might play a role [5]. Decreasing the pH of the incubation medium was found to result in a higher cell uptake of hematoporphyrin IX (HpIX) [5], chlorin e_6 [6] and merocyanine 540 (MC 540) [7]. The effect of pH on localization of photosensitizers in tumors has been confirmed also in several studies in vivo (e.g. [4,8]). The mechanism of the pH dependency of the photosensitizer uptake is not known. Evidence suggests that changes in the lipophilicity are involved in pH dependent drug uptake [9,10]. However, Lagerberg et al. [11] showed that the pH dependency of the uptake of MC 540 was explained by modification of the transmembrane potential of erythrocytes. Moreover, tumor cells as well as other proliferating cells were found to be depolarized when compared to normal non-proliferating cells [12].

The purpose of the present study was to examine whether pH dependent modification of transmembrane potential influences pH dependent cellular uptake of porphyrin-type photosensitizers. Therefore, the uptakes of three photosensitizers, hematoporphyrin IX (HpIX), disulfonated meso-tetraphenylporphine ($TPPS_{2a}$), meso-tetra(3-hydroxyphenyl)chlorin (mTHPC), by cells derived from solid tumors (WiDr, THX) and normal cells (skin fibroblasts) at two pH values that correspond to the pH in tumor (6.8) and normal tissue (7.4) were investigated in the absence of serum. The photosensitizers were chosen to have different acidity, which is directly related to ionic properties in this pH range. In addition, transmembrane potential of the cells was examined. A non-specific K^+ channel blocker, tetraethylammonium chloride (TEA), was used to test the contribution of transmembrane potential to cellular uptake of photosensitizers.

2. Materials and methods

2.1. Cells

The human cell lines: WiDr from colon adenocarcinoma, T47D from breast carcinoma, U87MG from malignant glioma and THX from malignant melanoma as well as normal human skin fibroblasts were used in the study. $5–7\times10^5$ (WiDr, T47D) or $1–1.5\times10^5$ (THX, skin fibroblasts) cells were inoculated in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin and 1% L-glutamine. 2×10^5 of U87MG cells were inoculated in EMEM medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin and streptomycin, 1% L-glutamine and non-essential amino acids. The cells were incubated in humidified 5% CO_2 atmosphere at 37 °C for about 48 h before experiments were performed.

2.2. Electrophysiological measurements

The cells ($\sim10^4$) were inoculated on glass coverslips (24 mm diameter) placed in small Petri dishes and incubated overnight before electrophysiological measurements. The coverslips were used as the floor of the experimental chamber on the stage of an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan). The cells were perfused (37 °C) with 0.05 M Tris–HCl buffers supplemented with salts to give the ionic composition corresponding to RPMI-1640 medium, namely 0.4 g/l KCl, 6 g/l NaCl, 0.1 g/l $Ca(NO_3)_2$, 0.1 g/l $MgSO_4\cdot7H_2O$, 2 g $NaHCO_3$ and $Na_2HPO_4\cdot2H_2O$, and adjusted to pH values 7.4 and 6.8 with HCl. pH 6.5 was used in the case of WiDr cells. In order to examine the contribution of a possible effect of Cl^- ions, the measurements on WiDr cells also were performed in buffers with pH adjusted with H_3PO_4. Cells were impaled with high-resistance microelectrodes pulled from borosilicated glass capillaries with inner filament (GC120F-10, Clark Electromedical Instruments, Pangbourne, UK) to a thickness that gave resistance of 50–80 MΩ when filled with 0.5 M KCl (corresponding to resistance of 19–25 MΩ when filled with 3 M KCl). High resistance microelectrodes were used to reduce cell dialysis. Recordings were made with an Axoclamp-2A amplifier (Axon Instruments, Foster City, CA, USA) in bridge-mode for current-clamp experiments using pCLAMP-8 software (Axon Instruments). The data were digitized with a Digidata 1200B and sampled and stored on a computer. Voltage recordings were analyzed with pCLAMP-8 software (Axon Instruments). Transmembrane potential was recorded within 200 ms after impalement to avoid the effect of cell dialysis.