FACTA UNIVERSITATIS
Series: **Medicine and Biology** Vol.9, No 1, 2002, pp. 70 - 73 UC 616.62-006

WILL HUMAN URINARY BLADDER CARCINOMA RESPOND TO TREATMENT WITH ALKYLPHOSPHOCHOLINES AND CURCUMIN?

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Summary. *Alkylphosphocholines (APC) constitute a new group of antineoplastic agents without hematological toxicity: their first clinically available derivative hexadecylphosphocholine (miltefosine) is locally used to control skin metastases of breast cancer. Curcumin is a natural product possesing many attractive pharmacological effects such as inhibition of inflammation, mutagenesis and cancerogenesis. Since intravesical chemotherapy represents a form of topical treatment we investigated whether new APC with long alkyl chain and the yellow non-toxic pigment curcumin would be active against 5637 and EJ bladder cancer cell lines. The antineoplastic activity was inversely related with the alkyl chain length of the respective APC. Erucylphosphocholine and its congener with modified phosphocholine head erucylphospho-N.N.N-trimethylpropanolamine were the most effective derivatives. Interestingly, curcumin caused equal cytotoxic efficacy. The distinct antineoplastic effects lead us to predict that urinary bladder instillation of APC or curcumin will be of therapeutic benefit for patients with urinary bladder neoplasias.*

Key words: *Bladder carcinoma cells, alkylphosphocholines, curcumin, erucylphosphocholine, topical treatment*

Introduction

Alkylphosphocholines (APC) constitute a new class of drugs which was originally derived from cytotoxic ether lipids (1,2). The best characterised representative of this class is hexadecylphosphocholine (HPC) which shows distinct antineoplastic activity in vitro (3,4,5) and in certain animal models (6,7). Its select activity against chemically induced rat mammary carcinoma was reason to initiate clinical trials in mammary carcinoma patients (8). These trials showed a particular activity against breast cancer skin metastases after topical administration. Topical treatment with HPC was also investigated in patients with malignant cutaneous disease such as lymphoproliferative disorders (9). Contrasting with the topical administration, systemic treatment failed to reach sufficient HPC levels due to gastrointestinal toxicity (10). Interestingly, the systemically achievable HPC levels are highly active against human leishmaniasis (11). The lack of measurable antineoplastic effects following systemic administration, however, favours the development of other local treatment modalities with APC. Topical chemothrapy with antineoplastic drugs is used e.g. for skin and urinary bladder neoplasias (12). Curcumin is the yellow pigment of radices from *Curcuma longa (L), Zyngiberaceae*. (13). Its anti-inflammatory, anti-oxidant, anti-mutagenic, hepatoprotective and hemostatic effects have been reported (14). Experiments in rats, dogs, monkeys and guinea pigs curcumin showed that curcumin did not cause any signs of toxicity at doses of 0.5 up to 2.0 g/kg (15). Moreover, curcumin has been described to possess anti-mutagenic and anti-cancerogenic properties (14).

Bladder cancer is the second most frequent urological malignancy corresponding to 2% of all human cancers. Its superfacial growth initially allows transurethral resection followed by intravesical chemotherapy to prevent tumor recurrence (16). In the present study we focused on the anticancer effects of new alkylphosphocholine analogues and the natural product curcumin in two human urinary bladder carcinoma cell lines. The aim of the investigation was to estimate the potential of APC and curcumin for local instillation treatment of patients with urinary bladder neoplasias.

Materials and methods

Compounds

The APC under investigation can be grouped according to their structure into those with typical choline polar head and saturated alkyl chain (dodecylphosphocholine, DPC, tetradecylphosphocholine, TPC, hexadecylphosphocholine, miltefosine, HPC; and octadecylphosphocholine, OPC); those with cyclic polar head and saturated alkyl chain (octadecyl-[2-(N-methylpiperidino)ethyl]-phosphate, OMPEP; and octadecyl-(1,1-dimethylpiperidino-4-yl)-phosphate, perifosine, ODPP), and those with typical or slightly modified choline polar head and unsaturated alkyl chain (erucylphosphocholine, EPC and

erucylphospho-N.N.N-trimethylpropanolamine, EPC3) (Fig. 1).

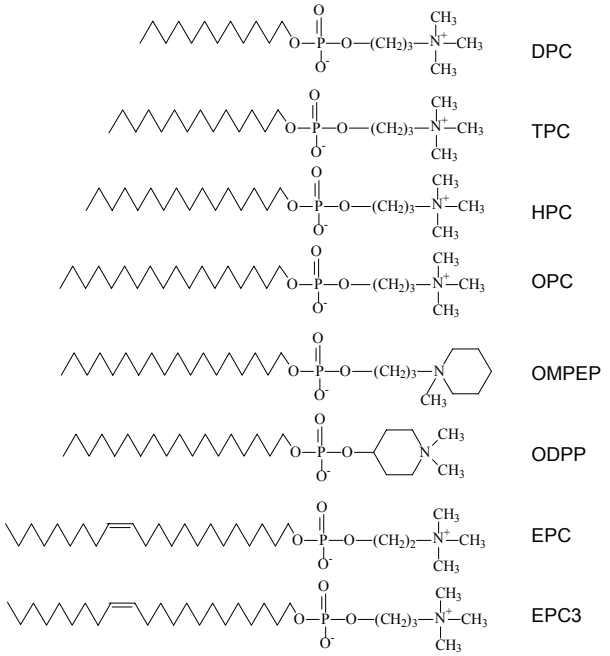

Fig. 1. Structure of alkylphosphocholines used

Curcumin as a pure substance was purchased from Sigma (C1386) (Fig. 2). Stock solutions (10 mM) were prepared and kept at –20°C in the dark until use.

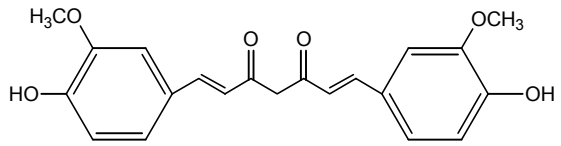

Fig. 2. Chemical structure of Curcumin

Cell lines and culture conditions

The two bladder carcinoma cell lines 5637 and EJ were grown as monolayer cell cultures (RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, 100 UI/ml penicillin and 100 µg/ml streptomycin) at 37°C in an incubator with humidified atmosphere and 5% CO_2. Cells were passaged by trypsinisation 2-3 times a week to keep them in log phase.

Cytotoxicity determination

Cells were seeded into 96-well plates (100 µl/well at a density of 1×10^5 cells/ml) and exposed to various concentrations of APC for 48 h or 72 h, as indicated below. The cell survival fraction was determined with the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide] dye-reduction assay as described by Mosmann (17), with some modifications. Briefly, after incubation with the test-compound, MTT-solution (10 mg/ml in PBS) was added (10 µl/well). Plates were further incubated for 4 h at 37°C and the formazan crystals formed were dissolved by adding 100 µl/well of 5% formic acid in 2-propanol. Absorption was measured by an ELISA reader (Anthos 2001) at 540 nm, reference filter 690 nm. For each concentration at least 8 wells were used. 100 µl RPMI 1640 medium with 10 µl MTT stock and 100 µl 5% formic acid in 2-propanol was used as blank solution.

Results

The results of investigating APC with increasing chain length (12-18 carbons) in two human urinary bladder carcinoma cell lines are given in Table 1. The cytotoxic profiles of APC consisting of an initial increase over control (see below), followed by a steep slope and a plateau like maximum effect can be better described by detailing both, IC_{50} and IC_{90} values. DPC, TPC, HPC, OPC and OMPEP caused 90% cell growth inhibition of 5637 cells at concentrations ranging from >90 µM (DPC) to 30 µM (OPC, OMPEP). The ratio of IC_{90} versus IC_{50} values was greater in more active APC with longer alkyl chain than in those with an alkyl chain below 16 carbons' length. Comparing the two cell lines, EJ cells were more resistant as shown by IC_{50} values ranging from 156 µM (TPC) to 60-70 µM (OPC, OMPEP) which are 40-60 fold higher than the respective values in 5637 cells. The third derivative with 18 carbons' chain length (ODPP, perifosine) was less active in 5637 cells, but more active in EJ cells than OPC and OMPEP. In addition, complete growth inhibition following ODPP was seen in both cell lines at 100 µM concentration (Fig. 3 a-b). The other two congeners with 22 carbons' chain length (EPC, EPC3) showed a comparable IC_{50} concentration (4-5 µM), but a distinctly lower IC_{90} (37-45 µM) and caused complete growth inhibition already at 50 µM (Fig. 3 c-f).

Table 1. IC50 and IC90 values (µM) estimated by MTT-assay after 72 h exposure to APC

Cell line	DPC	TPC	HPC	OPC	OMPEP	ODPP
5637						
IC_{50} (µM)	15	4	1.4	1.1	1.1	4.7
IC_{90} (µM)	>90	59	46	28	30	78.7
EJ						
IC_{50} (µM)	n.d.	156	85	61	66	5.7
IC_{90} (µM)	n.d.	>200	>150	>100	>100	89.3

The cytotoxic efficacy of curcumin in two human urinary bladder carcinoma cell lines is given in Table 2. Carcinoma cells showed remarkable sensitivity towards curcumin. At the higher investigated concentration (100 µM) the survival fraction was approximately 10% of the untreated control for both cell lines. Comparing the IC_{50} values EJ cells were found to be more sensitive towards curcumin (IC_{50} = 10.71 µM for 5637 cells and IC_{50} = 14.28 µM) (Table 2 and Fig. 4).

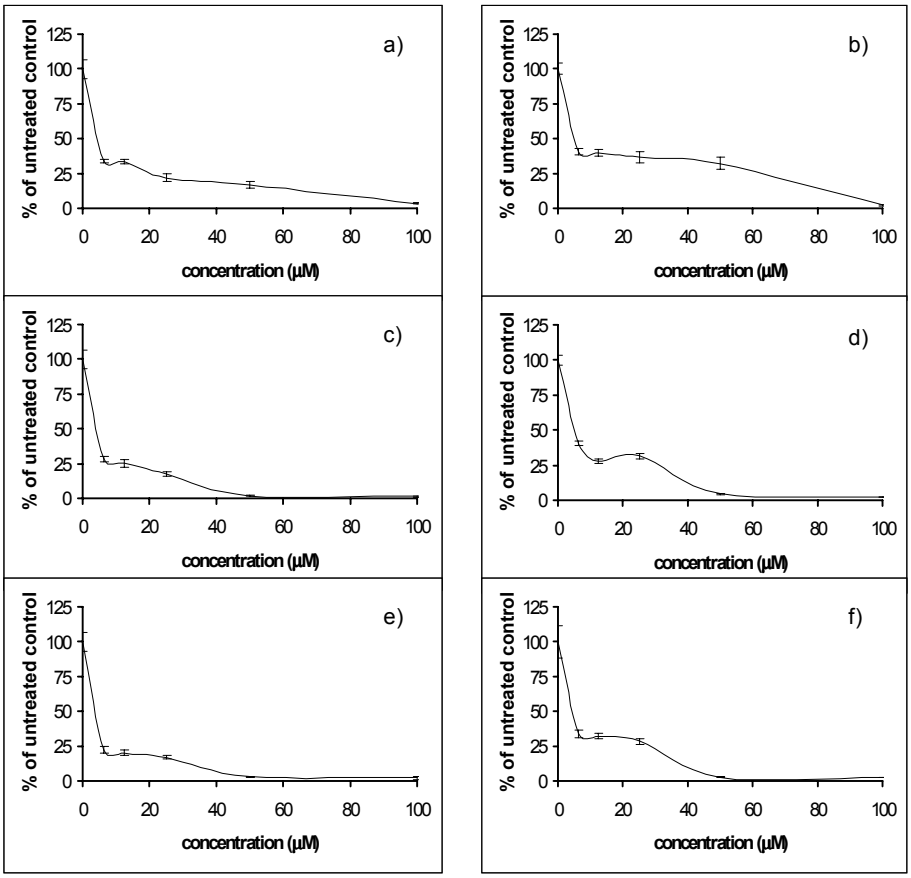

Fig. 3. Cytotoxic efficacy of octadecyl-(1,1-dimethylpiperidino-4-yl)-phosphate (Perifosine, ODPP) (a, b); erucylphosphocholine (EPC) (c, d) and erucylphospho-N,N,N-trimethylpropanolamine (EPC3) (e, f) against 5637 (a, c, e) and EJ (b, d, f) cells as measured by MTT-assay.

Table 2. IC_{50} and IC_{90} values (μM) estimated by MTT-assay after 48 h exposure to curcumin

Cell line	Curcumin
5637	
IC_{50} (μM)	14.28
IC_{90} (μM)	94.6
EJ	
IC_{50} (μM)	10.71
IC_{90} (μM)	>100

Discussion

Our experiments in two human urinary bladder carcinoma cell lines show that curcumin and all APC compounds investigated caused cell growth inhibition and cell death in a concentration dependent manner. The antineoplastic activity was inversely related with the alkyl chain length of APC. Thus, EPC and EPC3 were the most effective derivatives. These closely related compounds are characterised by an unsaturated alkyl chain which increase their water solubility over that of saturated analogues. They belong to the new group of i.v. injectable APC which combine a lack of hemolytic properties with an increased therapeutic index in MNU-induced rat mammary carcinoma (18). Comparing these two agents, EPC3 is similarly active but even more water soluble, which is advantageous for a potential clinical use. Curcumin was found to be equally effective on the tumor cell lines investigated. Our data about curcumin confirm the antineoplastic activity of curcumin

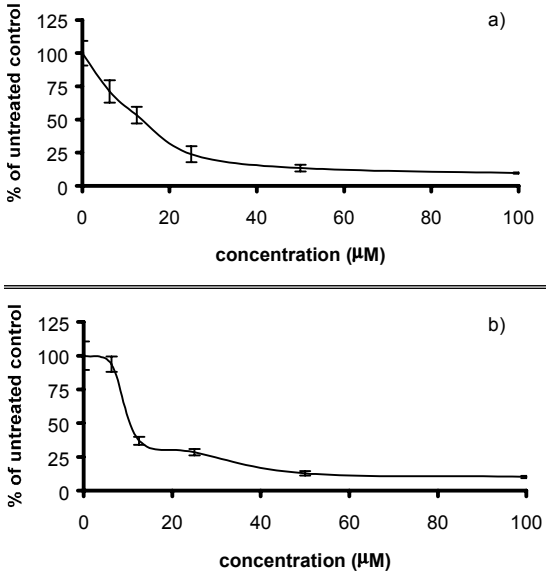

Fig. 4. Cytotoxic efficacy of curcumin against 5337 (a) and EJ (b) cells.

based on its ability to induce programmed cell death in human basal carcinoma cells causing a hyperexpression of p^{53} gene which is independent from the expression of other apoptosis inhibiting genes such as bcl-2 and bax (19). Investigations with MCF-7 mammary carcinoma cells treated with curcumin described some interesting aspects of its mode of action, e.g. inhibition of G_2 / M cell cycle progress (14).

The distinct antineoplastic effects observed in the two human urinary bladder carcinoma cell lines lead us to predict that urinary bladder instillation of APC or curumin will be of therapeutic benefit for patients with urinary bladder neoplasias. This prediction is made in the context that miltefosine was the first APC derivative which was registered for topical treatment of breast cancer skin metastases (20). Since urinary bladder neoplasias are currently treated by instillation of cytostatic and immunomodulating drugs such as anthracylines, mitomycin C, cisplatin, BCG and others (16), it seems promising to explore the potency of selected APC or curcumin for this indication. We expect that most of the drug will be retained by the urinary bladder wall. Even if the drug would be fully resorbed, adverse reactions are not expected since the resulting dosage is well below the maximum tolerated dose in humans (HPC: 100-200 mg/day) (21). Currently we don't know the effect of APC on normal urothelial cells, therefore we cannot exclude a certain degree of irritation due to a local high concentration of APC. Our knowledge, however, of APC effects on normal bone marrow and fibroblast cells gives us reason to assume, that urothelial cells will be not severely damaged (5). On the other hand, anthracyclines are known to cause local tissue damage (inflammation and necrosis), but they are nevertheless in clinical use. Selected APC derivatives such as EPC or EPC3 and curcumin could be suited candidates for local treatment of urinary bladder carcinoma patients in clinical trials.

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