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EFFECT OF URSODEOXYCHOLIC ACID ON VIABILITY AND PROLIFERATION OF HUMAN TUMOR CELLS

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ABSTRACT

PURPOSE: The aim of the study presented here was to evaluate the effect of ursodeoxycholic acid on viability and proliferation of cultured human tumor cells.

MATERIALS AND METHODS: The following permanent cell lines were included as model systems in the experiments: MCF-7 (human breast cancer), HeLa (human cervical cancer) and A549 (human lung cancer). The effects on cell viabilituy and proliferation were studied by MTT test and colony-forming method. Statistical differences between control and treated groups were assessed by unpaired Student *t*-test and calculated by Graph-Pad Prism 4.0 software package.

RESULTS: Applied at concentrations of 10, 50, 100 and 200 µg/mL for 24 h and 48 h, ursodeoxycholic acid (UDCA) decreased in a time- and concentration- dependent manner the viability of breast and lung cancer cells, while human cervical cancer cells remained almost unaffected. In the same concentration range (10-200 g/ml), UDCA did not inhibit completely the ability of tumor cells to grow in a semisolid medium.

CONCLUSION: Based on their sensitivity to the toxic effects of UDCA, the treated human tumor cell lines were graded as follows: MCF-7 > A549 > HeLa.

Kew words: Bile acids, Human tumor cell lines, MTT test, Colony-forming method

INTRODUCTION

Bile acids (BAs) are detergent molecules synthesized from cholesterol in the liver, that are released into the gut upon feeding and are essential for digestion. BAs are not only important for the absorption of dietary lipids and fat soluble vitamins but are signaling molecules with diverse endocrine and paracrine functions. They regulate lipid, glucose and bile acid metabolisms and modulate temperature and energy homeostasis. Bile acids can not only promote cell proliferation and liver regeneration but can also induce programmed cell death (1). Over the last decades the interest of scientists in BAs has grown markedly. Bile acids, their physiology and metabolism, their role in carcinogenesis and other major human diseases are recently undergoing significant progress (2, 3). As a result, bile acids have become increasingly important in a number of fields such as pharmacology, medicinal chemistry, biomimetic, supramolecular chemistry and also in nanotechnology. Pharmacological applications of bile acids and their derivatives are well known, including their use in the treatment of liver diseases, in dissolution of cholesterol gallstones, antiviral and antifungal properties. At the same time their potential to act as carriers of liver specific drugs and cholesterol level lowering agents has been reported (4). On the other hand, there are multiple epidemiologic data and scientific reports suggesting the role of bile acids in pathogenesis of human malignancies, especially those of the gastrointestinal tract (5, 6). In contrast, other studies have shown that

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bile acids exert cytostatic and cytotoxic effects in several human cell lines established from cancers of the breast (7), ovary (8), uterine cervix (9), prostate (10) and liver (11). Bile acids were also reported to inhibit angiogenesis in human hepatocellular carcinoma cells (12) and to induce differentiation in human acute promyelocytoc leukemia cells (13). The sum of these observations point to the necessity of further investigations in order to clarify better the biological actitvities of bile acids. The aim of the study presented here was to evaluate the effect of ursodeoxycholic (UDCA) acid on viability and proliferation of cultured human tumor cells.

MATERIALS AND METHODS

Chemicals:

Dimethyl sulfoxide (DMSO) and trypsin were purchased from AppliChem (Darmstadt, Germany). Ursodeoxycholic acid (Sigma Aldrich Chemie GmbH) was dissolved in DMSO (up to concentration of 10 μg/ml of the solvent) and then diluted in culture medium. The final concentration of DMSO in the stock solutions (where the concentration of the tested compound was 1 mg/mL) was 2%. Purified agar (Difco) and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemie GmbH (Germany). Dulbecco's modified Eagle's medium (D-MEM and fetal bovine serum were obtained from Gibco-Invitrogen (UK). Ethylendiaminotetraacetic acid (EDTA) and all other chemicals of the highest purity commercially available were purchased from local agents and distributors.

Cell cultures and cultivation:

The following permanent human cell lines were used as model systems in our study: MCF-7 (breast cancer), HeLa (cervical cancer) and A549 (lung cancer). The cells were grown as monolayer cultures in D-MEM medium, supplemented with 5-10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. The cultures were maintained at 37^{o}C in a humidified CO_2 incubator. For routine passages adherent cells were detached using a mixture of 0.05% trypsin (Gibco) – 0.02% ethylendiaminotetraacetic acid (EDTA). The experiments were performed during the exponential phase of cell growth.

Cytotoxicity assay:

The cells were seeded in 96-well flat-botommed microplates (Orange scientific) at a concentration of 2 x 10^4 cells/well. At the 24th h cells from monolayers were washed and covered with media modified with different concentrations of the compound tested. Each concentration was applied in 6 to 8 wells. Samples of cells grown in non-modified medium served as control. After 24 and 48 h of incubation, the solutions were removed from the plates and MTT colorimetric assay of cell survival was performed as described by Mossman (14). This consisted of three hours incubation with MTT solution (5 mg MTT in 10 mL D-MEM) at 37^{o}C under 5% carbon dioxide and 95% air; then extraction with a mixture of absolute ethanol and DMSO (1:1, vol/vol). The absorbance of each well at 540/615 nm was read by an automatic microplate reader (TECAN, SunriseTM, Grodig/Sazburg, Austria). Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration. All data points represent an average of three independent assays.

Colony-forming assay:

Tumor cells (approximately 10^3 cells/well) suspended in 0.45% purified agar in D-MEM medium containing different concentrations of the compounds examined (ranging from 10 to 200 μg/mL) were layered in 24 well microplates (Orange scientific). The presence/absence of colonies was registered using an inverted microscope (Carl Zeiss, Jena, Germany) during 14 days period.

Statistical analysis:

The data are presented as mean ± standard error of the mean. Statistical differences between control and treated cells were assessed by unpaired Student *t*-test and calculated by Graph-Pad Prism 4.0 software package.

RESULTS AND DISCUSSION

The effect of UDCA on cell viability and proliferation was studied by MTT test and colony forming method (CFM). Ursodeoxycholic acid was applied at a concentration range of 10-200 μg/mL for 24 and 48 h (MTT) or for 14 days (CMF). The results obtained are summarized in **Figures 1, 2 and 3.** Our data show that UDCA decreases in a time- and concentration- dependent manner the viability of the treated cells. The effect observed was found to be cell-specific: among the cell lines used as model systems in the experiments, the breast cancer (MCF-7) cells were relatively the most sensitive to the

action of UDCA, whereas HeLa remained almost unaffected ($P > 0.05$ for all concentrations and intervals of incubation). We did not find a concentration of UDCA in the range of 10 to 200 μg/mL, which completely inhibited the colony-forming ability of the tumor cells.

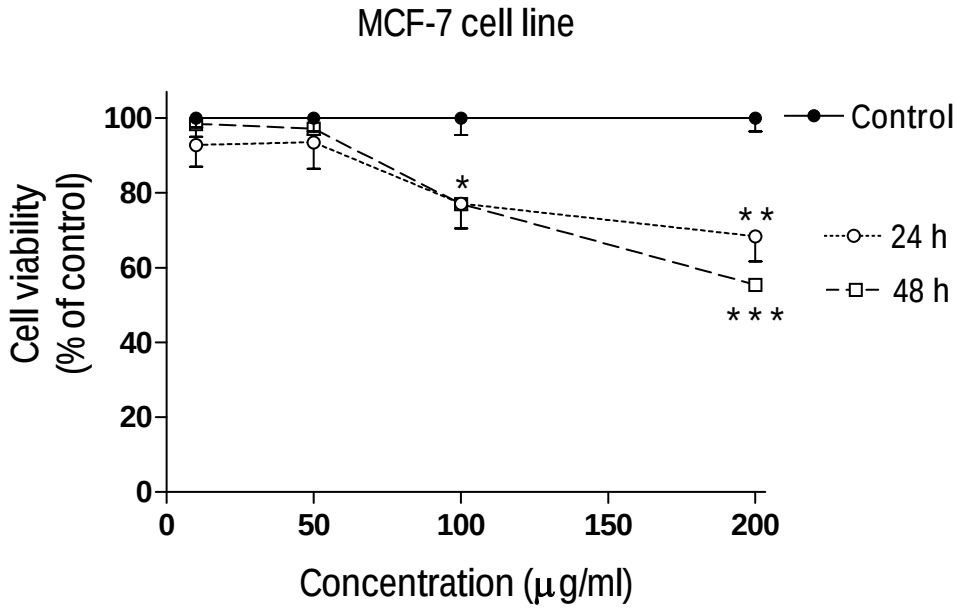

Figure 1. Viability of cultured human breast cancer cells (MCF-7) assessed by MTT test in controls (taken to be 100 %) and in the presence of 10, 50, 100 and 200 μg/ml of ursodeoxycholic acid, applied for 24 and 48 h. Means ± SEM are presented. Statistical analysis was performed by unpaired *t-test*, significant differences from the control: $*P<0.05$; $**P<0.01$; $***P<0.001$.

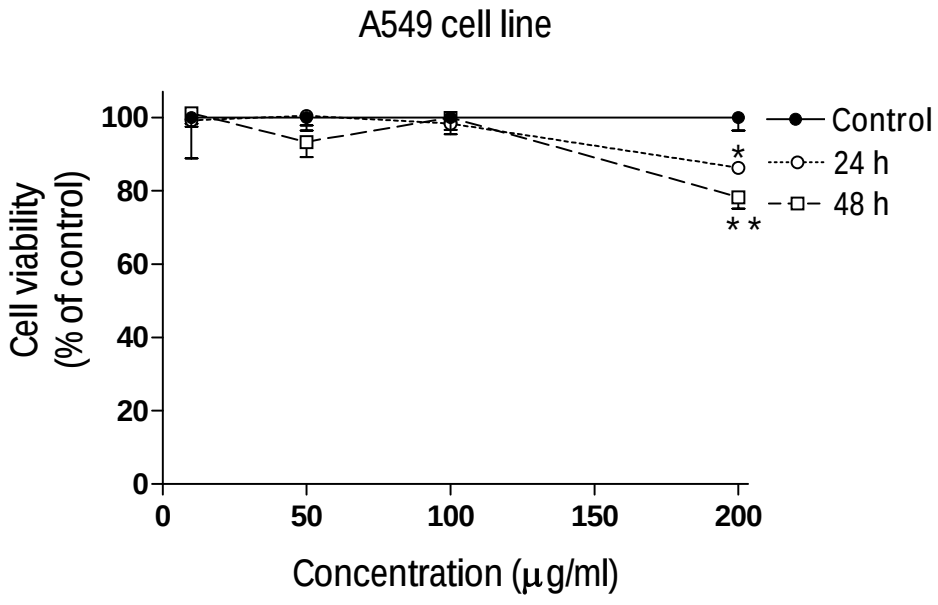

Figure 2. Viability of cultured human lung cancer cells (A549) assessed by MTT test in controls (taken to be 100 %) and in the presence of 10, 50, 100 and 200 μg/ml of ursodeoxycholic acid, applied for 24 and 48 h. Means ± SEM are presented. Statistical analysis was performed by unpaired *t-test*, significant differences from the control: $*P<0.05$; $**P<0.01$.

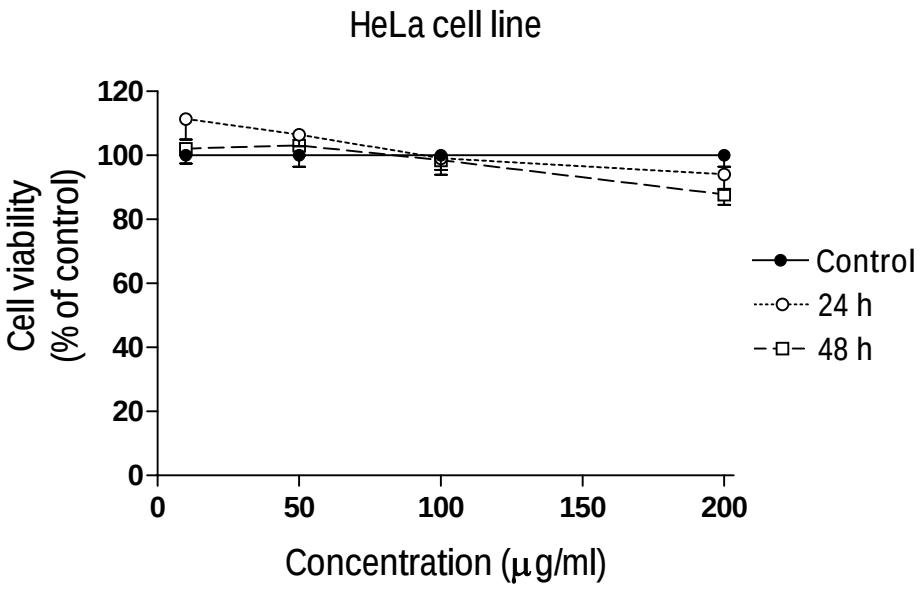

Figure 3. Viability of cultured human cervical cancer cells (HeLa) assessed by MTT test in controls (taken to be 100 %) and in the presence of 10, 50, 100 and 200 μg/ml of ursodeoxycholic acid, applied for 24 and 48 h. Means ± SEM are presented. Statistical analysis (differences from the control) was performed by unpaired *t-test* and calculated by Graph-Pad Prism 4.0 software package.

Bile acids are a group of molecular species of acidic steroids with peculiar physical chemical and biological characteristics. Primary BAs (such as cholic, chenodeoxycholic, etc) are directly synthesized from cholesterol by hepatocytes, by the addition of hydroxyl groups and the oxidation of its side chain to form more water soluble end product. The secondary bile acids (such as deoxycholic, lithocholic, ursodeoxycholic) are generated in the intestine by bacterial biotransformation of primary BAs (3, 15).

Depending on the nature of chemical structures, different bile acids exhibit distinct biological effects. Hydrophobic acids such as deoxycholic acid are known to be associated with carcinogenesis. In contrast, hydrophilic acids such as UDCA have protective effects such as prevention of gallstones through desaturation of bile and reduction of cellular changes seen in hepatic and biliary diseases (16-20).

It has been reported that UDCA has chemopreventive properties in both animal models (21-23) and humans (24). A randomized, placebo-controlled study has demonstrated that UDCA treatment for 6 months does not seem to induce changes in the proliferative behavior of the colorectal mucosa in patients with adenomas (25). Recent reports suggest that UDCA inhibits the initiation and postinitiation phases of azoxymethane-induced colonic tumor development (26) and reduces hepatocancerogenesis in rats (20). There are data that UDCA may act to suppress cell growth by inhibiting the mitogenic activity of receptor tyrosine kinase such as EGFR through increased receptor degradation (27). The ability of ursodeoxycholic acid to inhibit proliferation and induce apoptosis of HepG2 and BEL7402 hepatocellular cancer cell lines by blocking cell cycle and regulating the expression of Bax/bcl-2 genes has also been reported (11).

In conclusion, based on their sensitivity to the toxic effects of UDCA, the treated human tumor cell lines in our experiments were graded as follows: MCF-7 > A549 > HeLa. At the same concentration range (10-200 g/ml), UDCA did not inhibit completely the ability of tumor cells to grow in a semisolid medium.

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