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EFFECT OF URSODEOXYCHOLIC AND DEOXYCHOLIC ACIDS ON THE VIABILITY AND PROLIFERATION OF VIRUS-TRANSFORMED TUMOUR CELLS

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Abstract

The aim of the present study was to evaluate the effects of ursodeoxycholic (UDCA) and deoxycholic (DCA) acids on the viability and proliferation of cultured virus-transformed tumour cells. The following permanent cell lines were used as model systems in our study: LSCC-SF-Mc29 (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29) and LSR-SF-SR (transplantable sarcoma in a rat induced by Rous sarcoma virus strain Schmidt-Ruppin). The investigations were performed by MTT test, neutral red uptake cytotoxicity assay and colony-forming method. The compounds tested were applied at a concentration range of 10–200 µg/ml for 24, 48 and 72 h. The results reveal that UDCA and DCA decrease significantly the viability and proliferation of the treated cells in a time- and concentration-dependent manner.

Key words: ursodeoxycholic acid, deoxycholic acid, cytotoxic activity, virus-transformed tumour cells

Introduction. Bile acids (BAs) are a group of molecular species of acidic steroids with peculiar physical, chemical and biological characteristics. Primary BAs (such as cholic and chenodeoxycholic) 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 intestines by bacterial biotransformation of primary BAs [1, 2].

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In the recent years steroidal structures became increasingly important in a number of fields such as pharmacology, medicinal chemistry, biomimetic and supramolecular chemistry and also in nanotechnology. There are well known pharmacological applications of bile acids and their derivatives, including their use in the treatment of liver diseases, in dissolution of cholesterol gallstones, as well as their potential to act as carriers of liver specific drugs and cholesterol level lowering agents [3]. At the same time, 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 [4]. In contrast, other studies showed that bile acids exert cytostatic and cytotoxic effects in several human cell lines established from cancers of the breast [5], ovary [6], uterine cervix [7], prostate [8], liver [9], etc. Bile acids were also reported to inhibit angiogenesis in human hepatocellular carcinoma cells [10] and to induce differentiation in human acute promyelocytoc leukaemia cells [11]. The sum of these observations points to the necessity of further investigations to clarify better the biological activities of bile acids.

The aim of the presented study was to evaluate the effect of the secondary bile acids – ursodeoxycholic (UDCA) and deoxycholic (DCA) acids on the viability and proliferation of cultured retrovirus-transformed tumour cells.

Materials and methods. *Chemicals.* Dimethyl sulfoxide (DMSO) and trypsin were purchased from AppliChem (Darmstadt, Germany). Ursodeoxycholic acid (Sigma Aldrich Chemie GmbH) and deoxycholic acid (Calbiochem) were dissolved in DMSO (up to concentration of 10 mg/ml) and then were diluted in the 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 obtained from Sigma Aldrich Chemie GmbH (Germany), Dulbecco's modified Eagle's medium (D-MEM and fetal bovine serum were 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 two cell lines were used as model systems in our study: LSCC-SF-Mc29 (established from transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29) [12], and LSR-SF-SR (derived from transplantable sarcoma in a rat induced by Rous sarcoma virus strain Schmidt-Ruppin) [13]. 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 °C in a humidified CO_2 incubator. For routine passages adherent cells were detached using a mixture of 0.05% trypsin (Gibco) and 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-bottomed microplates (Orange scientific) at a concentration of 2×10^4 cells/well. At the 24th hour the

cells from the monolayers were washed and covered with media modified with different concentrations of the compounds tested. Each concentration was applied in 6 to 8 wells. Samples of cells grown in non-modified medium served as control. After 24, 48 and 72 h of incubation, the solutions were removed from the plates and the cell viability was examined by MTT test [14] and neutral red uptake cytotoxicity assay (NR) [15]. The optical density was measured at wave length 540 nm by automatic microplate reader (TECAN, Sunrise$^{\text{texttrademark}}$, Grodig/Salzburg, Austria). The relative cell viability, expressed as 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. Tumour 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 a 16-day period.

Statistical analysis. Data were 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. We summarized the results obtained by MTT test (Figs 1 and 3) and those obtained by NR test (Fig. 2). In our experiments the compounds tested (UDCA and DCA) were applied at concentrations of 10, 50, 100 and 200 µg/ml for 24, 48 and 72 h.

The data about the influence of UDCA and DCA on the ability of tumour cells to form colonies in semisolid medium are presented in Table 1.

The results obtained by us revealed that UDCA and DCA decreased significantly the viability and proliferation of cultured chicken hepatoma and rat

T a b l e 1

Effects of ursodeoxycholic (UDCA) and deoxycholic (DCA) acids on the colony-forming ability of virus-transformed tumour cells

Compound	Concentration (µg/ml)			
	10	50	100	200
LSR-SF-SR				
UDCA	+	+	+	+
DCA	+	+	+	+/−
LSCC-SF-Mc29				
UDCA	+	+	+	+
DCA	+	+	+/−	−

(+) Presence of colonies
(−) Absence of colonies
(+/−) Presence of single colonies

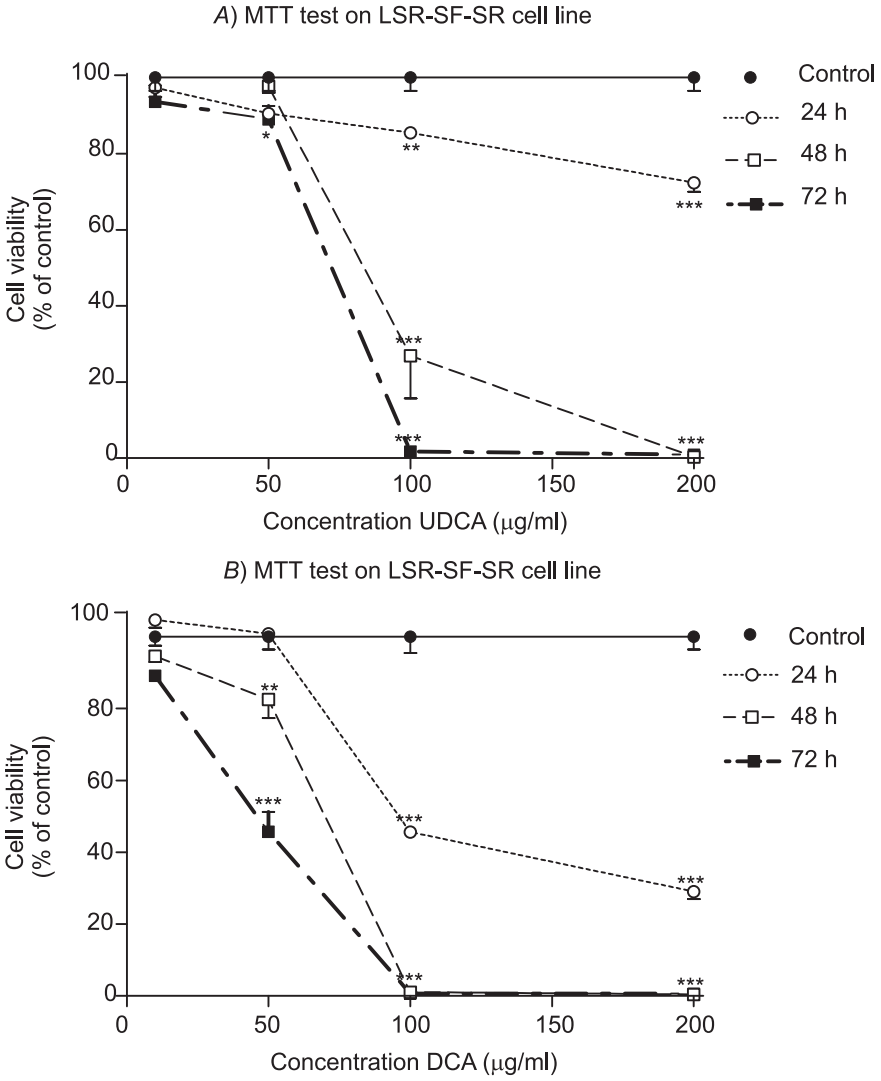

Fig. 1. Viability of cultured LSR-SF-SR virus-transformed tumour cells (transplantable sarcoma in a rat induced by Rous sarcoma virus strain Schmidt-Ruppin) assessed by MTT test in controls (taken to be 100%) and in the presence of 10, 50, 100 and 200 μg/ml of: *A*) ursodeoxycholic acid (UDCA) and *B*) deoxycholic acid (DCA) applied for 24, 48 and 72 h. Means ± SEM were presented. Statistical analysis was performed by unpaired *t*-test, significant differences from the control: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

sarcoma cells in a time- and concentration-dependent manner. Good correlations between data coming from short-term tests (NR and MTT, 24–72 h) in monolayer cultures and long-term colony-forming assay (16 days, 3D colonies in semi-solid medium) as well as between MTT (reflects damage to mitochondria) and NR (indicates damage to lysosomes and Golgi apparatus) methods were observed.

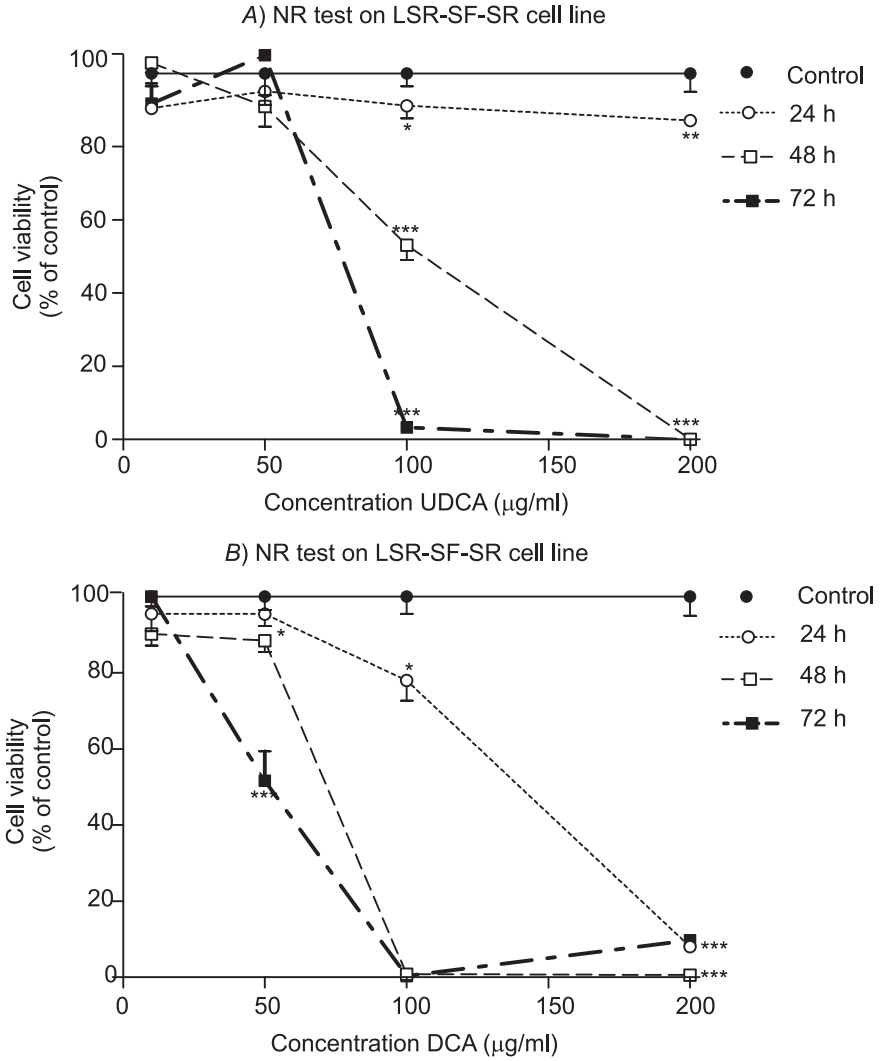

Fig. 2. Viability of cultured LSR-SF-SR virus-transformed tumour cells (transplantable sarcoma in a rat induced by Rous sarcoma virus strain Schmidt-Ruppin) assessed by NR test in controls (taken to be 100%) and in the presence of 10, 50, 100 and 200 μg/ml of: *A*) ursodeoxycholic acid (UDCA) and *B*) deoxycholic acid (DCA) applied for 24, 48 and 72 h. Means ± SEM were presented. Statistical analysis was performed by unpaired *t*-test, significant differences from the control: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

We decided to use as model systems the cell lines LSCC-SF-Mc29 and LSR-SF-SR because of the following main reasons: i) according to the literature available, the effects of UDCA and DCA on the viability and proliferation of retrovirus-transformed cells have not been evaluated yet; ii) the cells of both lines carry specific viral oncogenes – v *myc* (in the case of LSCC-SF-Mc29) and v-*src* (in the

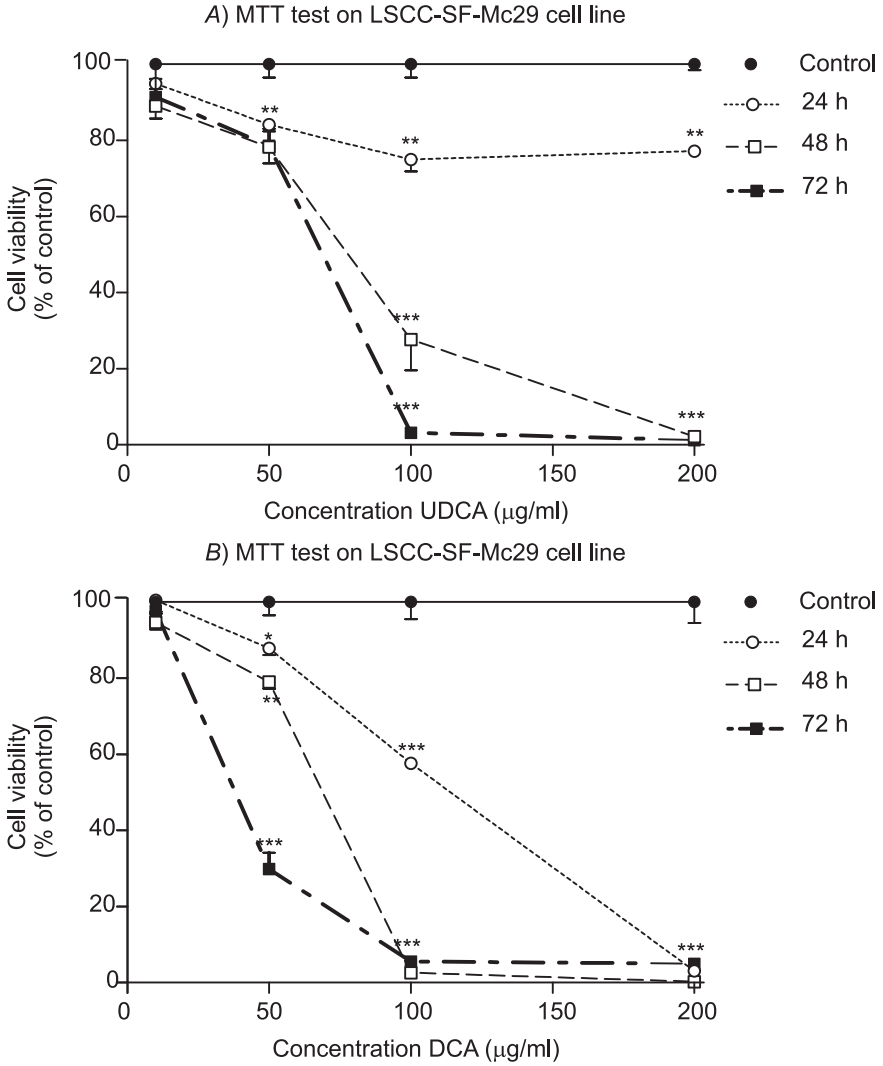

Fig. 3. Viability of cultured LSCC-SF-Mc29 virus-transformed tumour cells (transplantable chicken hepatoma induced by the myelocytomatosis virus Mc29) assessed by MTT test in controls (taken to be 100%) and in the presence of 10, 50, 100 and 200 μg/ml of: *A*) ursodeoxycholic acid (UDCA) and *B*) deoxycholic acid (DCA) applied for 24, 48 and 72 h. Means ± SEM were presented. Statistical analysis was performed by unpaired *t*-test, significant differences from the control: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

case of LSR-SF-SR). It is well known that *myc* (*c-myc*, *L-myc*, *N-myc*) and *src* proto oncogenes are involved in pathogenesis of a wide variety of malignancies in humans; iii) these cell lines are successfully used in our previous investigations to evaluate the putative antitumour activity of alkaloids and metal complexes with various ligands [12].

Over the last decades the interest of scientists in bile acids has grown markedly. Bile acids, their physiology and metabolism, their role in carcinogenesis and other socially important human diseases are recently undergoing significant progress. Depending on the nature of chemical structures, different bile acids exhibit distinct biological effects. It has been suggested that DCA is implicated in various cancers of the gastrointestinal tract [4]. On the other hand, DCA can induce apoptosis in the human colon cancer cell line HCT116 in the absence of Bax [16], it can inhibit growth and induce apoptosis of esophageal cancer Eca109 cells [17]. Deoxycholic acid-modified carboxymethyl curdlan conjugate has been suggested to be a novel carrier of epirubicin in in vitro and in vivo experiments [18]. Recent reports suggest that UDCA inhibits the initiation and postinitiation phases of azoxymethane-induced colon tumour development [19] and reduced hepatocancerogenesis in rats [20].

Further experiments are needed to clarify better the relationship between ursodeoxycholic and deoxycholic acids and cancer as well as to evaluate the putative antineoplastic activities of these bile acids and their derivatives.

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