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Antiproliferative and Antimigratory Effects of Doxorubicin in Human Osteosarcoma Cells Exposed to Extracellular Matrix

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Abstract. *Osteosarcoma cells are involved in the remodeling of the extracellular matrix (ECM) that affects their growth, invasive and metastatic activities. The tumour ECM provided effective protection against chemotherapy agents in several previously studied malignancies. The current study examined the effects of doxorubicin on cells that were migrated into a 3-dimensional extracellular matrix gel (ECM-gel) in comparison with its effects on cells remaining in the monolayer compartment. A human osteosarcoma cell line (OSCORT) was treated with doxorubicin in monolayer culture for 4 or 24 hours, and then overlaid by ECM-gel for 24 hours. Tumour cells remaining in the monolayer were separated from the cells migrated into ECM-gel, and both of them were characterized. OSCORT cells migrated into ECM-gel showed elevated levels and activity of topoisomerase II, increased protein expression of β1 integrin and matrix metalloproteinase-9 activity. Doxorubicin treatment for 4 hours resulted in increased cytotoxicity in the monolayer compartment relative to the cells migrated into ECM-gel, whereas 24-hour treatment at a low concentration (0.01 μg/ml) showed an antimigratory effect. Different antiproliferative and antimigratory effects of doxorubicin treatment schedules warrant short-term, high-dose treatment for targeting the tumour growth, and long-term, low-dose treatment for targeting the invasion of osteosarcoma.*

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The extracellular matrix (ECM) is a framework of proteins and proteoglycans, secreted by surrounding stromal fibroblasts. The ECM gives structural integrity to tissues. Structural changes in the ECM are necessary for cell migration during normal and pathologic tissue remodeling and neoplastic cell invasion (1, 2). When the normal ECM is replaced by a tumour matrix, the most prominent changes are: more than 75% loss of laminin, collagen type IV and E-Cadherin, and new deposits of collagen type I and type III in the mesenchyme. Collagen IV expression was positively correlated with the degree of tumour cell differentiation. Higher expression of matrix metalloproteinases (MMPs) was associated with severe symptoms, increased tumoural size and worse grade (3-6). Matrix metalloproteinases are a family of zinc-dependent enzymes, which degrade various components of the ECM and play an important role in facilitating tumour cell invasion. The family includes the gelatinases, stromelysins and collagenases. MMPs and their inhibitors have been identified as critical modulators of ECM composition and are thus crucial in neoplastic cell progression, invasion and metastasis (1, 2, 7-9).

Osteosarcoma cells are able to produce ECM components; flow cytometric measurements of total cellular amounts from human xenografts, spheroids and monolayer cultures showed that cells from xenografts usually contained collagen I, fibronectin, acetylglucosamine and acetylgalactosamine equal to or greater than those in the spheroid cells, which contained amounts equal to or less than those in the monolayer cells (10). On the other hand, osteosarcoma cells might produce a wide spectrum of MMPs, including MMP-1, 2, 3 and 9. The same osteosarcoma cells produce different amounts and kinds of enzymes involved in local infiltration, or remote metastases, and may increase the production of the enzymes most required under specific environments (11, 12). Thus, in the case of osteosarcoma, biological features such as

differentiation, proliferation, viability, migration (13,14), as well as the responses to therapeutic agents cannot be examined without taking the interactions between the cells and ECM into consideration. Several studies have shown that the ECM reduces chemotherapeutic drug-induced apoptosis in small cell lung cancer cells, myelomas and gliomas (15).

Our previous study used extracellular matrix gel (ECM-gel) to provide a matrix-based culture system as an alternative to the conventional plastic cell culture, and showed a protective effect for the matrix-anchored cells against cytotoxic damage (16). Osteosarcoma is usually treated with intensive preoperative and postoperative chemotherapy and wide tumour resection, resulting in a 60% to 70% 5-year survival rate. Doxorubicin (Doxo) is also included in this treatment schedule (17). In a previous study, the protection effect of stromal ECM for colon cancer cell lines against the cytotoxity of topoisomerase (topo) poisons such as etoposide or camptothecin was detected, but could not be correlated with increased expression of antiapoptotic proteins, like bcl-2 and bcl-x_L. In this form, the mechanism of this protection effect remained unexplained (15). Another study by Rintoul and Sethi had more success in revealing the mechanism of the ECM protection against the cytotoxic effect of topoisomerase inhibitors, showing that adhesion of small cell lung cancer cells to fibronectin, laminin and collagen IV through β1 integrins conferred resistance to apoptosis induced by standard chemotherapeutic agents, including etoposide, cisplatin and doxorubicin. The authors proved that adhesion to ECM proteins stimulated protein tyrosine kinase (PTK) activity in both untreated and etoposide-treated cells, which hindered caspase-3 activation, a convertase enzyme involved in the apoptosis (18).

In the present study, an osteosarcoma cell line (OSCORT) was used for comparison of the antiproliferative and antimigratory effects of doxorubicin between cells upwards-migrated into a 3-dimensional ECM-gel and cells remaining in the proliferating monolayer compartment. It was found that migration of osteosarcoma cells into the ECM-gel was accompanied by an elevation of topoisomerase II activity, the target molecule for doxorubicin.

Materials and Methods

Chemicals. Cell culture media reagents and chemicals of analytical grade were all purchased from Sigma (St. Louis, MO, USA), Merck (Darmstadt, Germany), Boehringer Ingelheim (Heidelberg, Germany) and Boehringer Mannheim (Mannheim, Germany). Doxorubicin was a gift from Pharmacia Kft. (Budapest, Hungary). The antibodies are specified in the corresponding sections.

OSCORT cell line, culture conditions and doxorubicin treatment. Osteosarcoma cell line (OSCORT) has been established in our laboratory from a primary osteosarcoma developed in the humerus of a young male. The characteristic feature of the *in vivo* xenograft and the *in vitro* cell culture designated as OSCORT, including its human origin and osteoid markers, will be published separately (manuscript in preparation by R. Harisi, M. Szendroi, F. Timar, A. Jeney, 2005).

7 x 10^4 OSCORT cells / cm^2 were cultured on 24-well plates (Greiner, Nürtingen, Germany) for 24-72 hours in RPMI-1640 medium supplemented with 10% fetal calf serum plus penicillin (100 unit/ml medium) and streptomycin (100 μg/ml medium), at 37˚C, in a humidified atmosphere containing 5% CO_2. Treatment with various doxorubicin concentrations was performed in monolayer for 4 or 24 hours. Then the medium was removed from both treated and untreated cultures, the cells were washed and the ECM (10 mg/ml in RPMI-1640 medium + 10% fetal calf serum) was overlaid. At the end of the 24-hour incubation period, the ECM-gel with the migrating cells was separated mechanically from the monolayer. The gel was digested with 2.5 mg/ml dispase (Boehringer Ingelheim) for 20 minutes at room temperature, and the digestion terminated by 53.7 μmol/l EDTA for cell counts or for further studies. The number of cells, released from the ECM-gel by gentle mechanical stirring, and from the monolayer with trypsin-EDTA treatment, was counted in a haemocytometer and processed for flow cytometry, enzymatic measurements and immunocytochemical study.

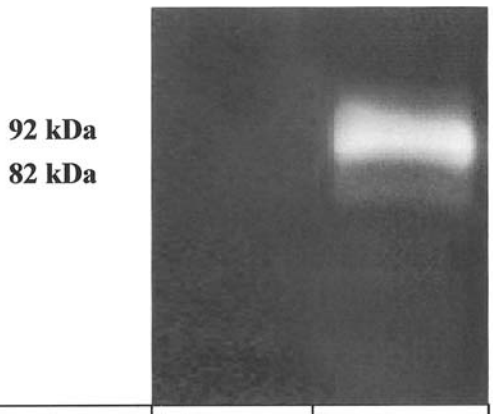
Preparation of ECM. ECM extract (ECM-gel) was isolated from Engelbreth-Holm-Swarm (EHS) sarcoma, as described previously (16, 19-21).

Flow cytometry analysis. Cell cycle parameters were studied as reported previously (22). Briefly: 10^6 /ml OSCORT cells were suspended in 0.1% sodium citrate, 0.1% Triton X-100, 0.05 mg/ml RN-ase, pH 7.3, incubated and stained with 50 μg/ml propidium iodide for 20 minutes. Flow cytometry was performed with FACScan (Becton-Dickinson Immunocytometry Systems, San Jose, CA, USA). The data were evaluated by using Rabinovitch's Multicycle software (Phoenix Flow Systems Inc., San Diego, CA, USA).

Detection of metalloproteinase activity. MMP activity was detected from the serum-free conditioned medium corresponding to 10^5 OSCORT cells per sample, as described previously in detail (22-24).

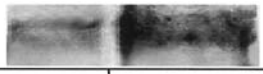
Immunoblot analysis. To identify β1 integrin and topoisomerase II-α proteins, OSCORT cells were disrupted in hypotonic medium in a Potter homogenizer. Nuclear and cytoplasmic fractions were separated after centrifugation at 3000 rpm and the protein concentration was determined. Cell lysates, cytoplasmic or cell nuclear extracts were analysed on sodium dodecyl sulfate - 10% polyacrylamide gel electrophoresis (SDS-PAGE), corresponding to 10^5 cells, and electrotransferred to nitrocellulose membranes (BioRad, Hercules, CA, USA). Antibodies were used in dilutions as follows: β1 integrin (clone JB1a): 1:300, mouse monoclonal (Chemicon, Temecula, USA); topoisomerase II-α: 1:500 rabbit polyclonal (Novocastra Laboratories Ltd., Newcastle upon Tyne, UK). Immunoblots were developed with chemiluminescence (Amersham, Buckinghamshire, UK) and densitometred with Eagle Eye II still video system (Stratagene, La Jolla, CA, USA).

Immunocytochemistry. Cytospin cell specimens were prepared from the OSCORT culture and fixed for 15 minutes with methanol and for 3 seconds with acetone, blocked with 5% bovine serum albumin in phosphate-buffered saline (PBS) containing 0.5% Triton X-100, 20 mM NH_4OH (pH=6.5). Primary antibody [anti-topoisomerase II



Matrix metalloproteinase activity	Plastic	ECM-gel
	Integrated optical density / 10^5 cells	
92 kDa	0.000	0.846
82 kDa	0.000	0.233

A.



β1 integrin protein expression	Plastic	ECM-gel
	Integrated optical density / 10^5 cells	
p 130	2.974	11.898

B.

Figure 1. *MMP activity and β1 integrin protein expression in OSCORT tumour cells exposed to ECM-gel. A. OSCORT cells were cultured with or without ECM-gel and matrix metalloproteinase activity was detected. Plastic: MMP activity in cells cultured on plastic dish; ECM-gel: MMP activity in cells cultured on ECM-gel B. Immunoblot detection of β1 integrin in OSCORT cells cultured on plastic surface or on ECM-gel. OSCORT cells were cultured with or without ECM-gel and integrin expression was detected. Plastic: β1 integrin protein expression in cells cultured on plastic dish; ECM-gel: β1 integrin protein expression in cells cultured on ECM-gel. Immunoblots were analysed with densitometry.*

rabbit polyclonal (TopoGen, Columbus, Ohio, USA)] was used overnight in 1:200 dilutions. The primary antibody was detected by biotinylated universal anti-mouse, anti-rabbit secondary antibody (Vector, Burlingame, CA, USA) and streptavidin-fluorescein (Amersham, Buckinghamshire, UK). Samples were analysed using the BioRad MRC 1024 confocal laser-scanning microscope (BioRad).

Topoisomerase II activity measurements. Cell nuclear extracts were prepared from cultured cells, according to the method reported by Dignam *et al.* (25). Briefly, cells were homogenised in a hypotonic buffer: 10 mM Tris-HCl (pH=7.5), 1.5 mM $MgCl_2$, 10 mM NaCl, 1% NP-40. The homogenate was centrifuged at 600 x g, for 10 minutes at 4˚C, the pellet was resuspended in TKCM buffer [50 mM Tris-HCl (pH=7.5), 25 mM KCl, 2 mM $CaCl_2$, 5 mM $MgCl_2$] and sedimented in sucrose gradient (0.25M to 0.6M) by centrifugation at 1000 x g for 10 minutes at 4˚C. Cell nuclear proteins were extracted in 0.35M NaCl containing TKM buffer [50 mM Tris-HCl (pH=7.5), 7.5 mM KCl, 1.5 mM $MgCl_2$] by 60-minute incubation on ice, followed by centrifugation at 12000 x g for 30 minutes.

The catalytic activity of topoisomerase II was assayed using the decatenation assay as described previously (26). Nuclear extract of 2.5 x 10^4 OSCORT cells was suspended in 50 mM Tris-HCl (pH=7.5), 85 mM KCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.5 mM EDTA, 0.03 mg/ml bovine serum albumin -1 mM ATP- and incubated with 200 ng kinetoplast DNA (kDNA) for 30 minutes at 37˚C. The reaction was terminated by the addition of 3% SDS – 1 mg/ml proteinase K (final concentration) and bromophenol-blue-TBE-glycerol at 50˚C for 30 minutes. Samples were then electrophoresed in 1% agarose gel using TBE buffer [89 mM Tris-borate, 2 mM EDTA (pH=8.3)] at 35 V for 4 hours. Gels were stained with ethidium bromide (1 μg/ml) and differentiated in H_2O. DNA bands were visualised and analysed in a Stratagene Eagle-Eye II video densitometer.

To detect the cleavage capacity of topoisomerase II, pBR322 plasmid was 3'end-labelled with α^{32}P dATP, and 2 x 10^4 dpm of it was used in the same reaction mixture as for the decatenation assay, but with 20 times more nuclear protein and with 100 μmol/l etoposide as specific inhibitor of topoisomerase II (27). The DNA cleavage activity of topoisomerase II is followed by religation of the double-stranded breaks; consequently, cleavage can only be visualised with etoposide, which specifically binds to DNA-topoisomerase II cleavable complex and freezes it. The reaction was initiated by the addition of nuclear extracts and terminated by addition of 3% SDS – 1 mg/ml proteinase K. The resulting double-stranded breaks were detected with electrophoresis in 1% agarose at 20 V for 16 hours, and then the gels were dried, followed by overnight autoradiography using Kodak X-Omat (Rochester, NY, USA) films. The cleavage method is an alternative for determining topoisomerase II activity.

Statistics. A significant difference between experimental data was stated when the *p*-value by the Student's *t*-test was under 0.05.

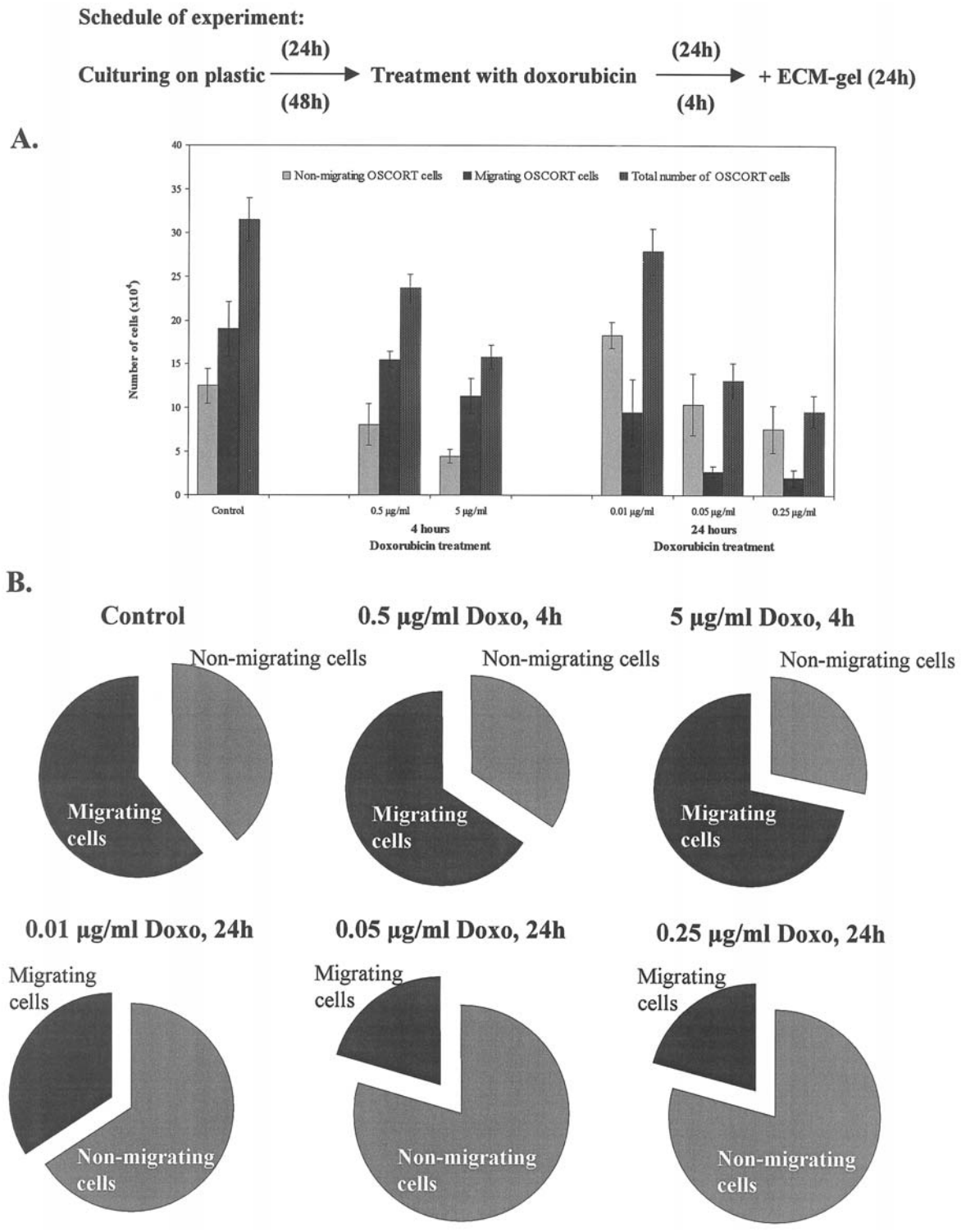


Figure 2. *Effect of 4- and 24-hour doxorubicin treatment on the proliferation and migration of OSCORT cells. In the uper panel of the figure, the summary of the experimental schedule is explained. Cells were cultured on plastic cell culture dishes for 24 or 48 hours, then treated with 0.5 and 5.0 µg/ml doses of doxorubicin for 4 hours, or with 0.01, 0.05 and 0.25 µg/ml doses for 24 hours. Then, the cells were overlayid by ECM-gel for 24 hours, and the cell numbers in the ECM-gel-migrating and the remaining non-migrating compartments were determined. (The duration of culture of the cells on plastic dish without doxorubicin treatment, before overlaying of the ECM-gel had no significant effect on the cell numbers) A. Comparison of total cell numbers, migrating and non-migrating cell numbers after doxorubicin treatment. B. Comparison of the distribution of cells between the migrating and non-migrating compartments after short-term (4h) and long-term (24h) doxorubicin (Doxo) treatments.*

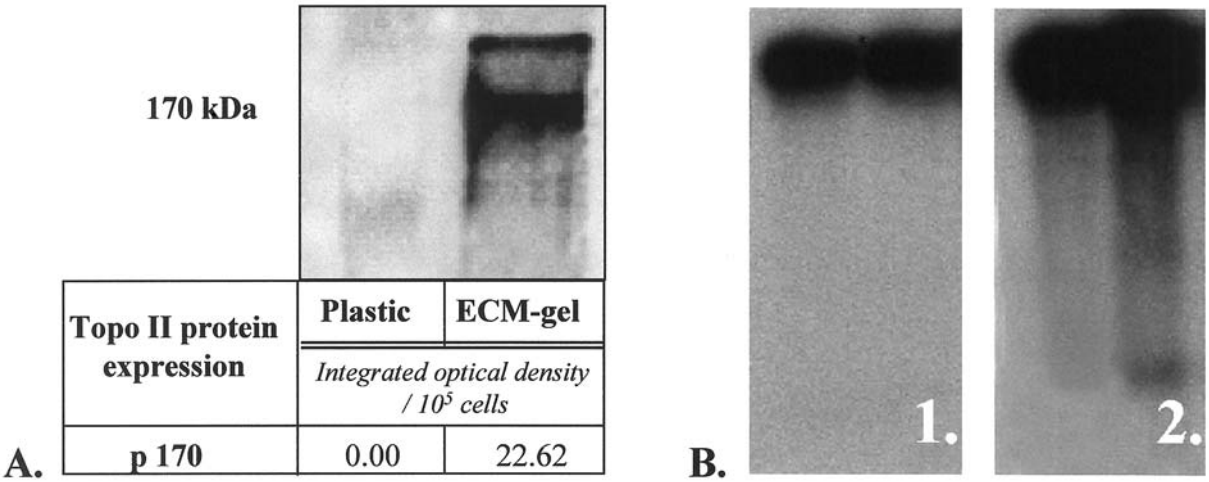


Figure 3. *Comparison of protein expression and cleavage activity of topoisomerase II in OSCORT cells cultured on plastic surface or on ECM-gel. A. Protein expression: OSCORT cells were cultured with or without ECM-gel and topoisomerase II protein expression was detected in cell nuclear extracts of 10^5 OSCORT cells. Plastic: Topoisomerase II expression of cells grown on a plastic tissue culture dish; ECM-gel: Topoisomerase II expression of cells grown on ECM-gel. B. Cleavage activity: Since topoisomerase II-mediated DNA cleavage is followed by a religation step of the double-stranded breaks, in order to detect the cleaved DNA it was necessary to trap the DNA – topoisomerase II complexes with etoposide (a structure-specific agent that freezes this cleavable complex). OSCORT cells were cultured with or without ECM-gel and the cleavage activity of topoisomerase II was measured. The extent of etoposide-trapped cleavage indicates topoisomerase II activity. B1. Cleavage activity in cell nuclear extracts from 5 x 10^5 cells cultured on plastic tissue culture dish: lane on the left, without etoposide; lane on the right, with 100 μM etoposide given to reaction mixture. B2. Cleavage activity in cell nuclear extracts from 5 x 10^5 cells cultured on ECM-gel: lane on the left, without etoposide; lane on the right, 100 μM etoposide was given to reaction mixture.*

Table I. *Cell population kinetic changes after treatment with doxorubicin.*

Cell source	Treatment	Cell cycle distribution of cells in %		
		G1	S	G2
Non-migrating	Not treated	73.4±2.3	9.4±2.0	16.4±1.4
	Doxorubicin 0.5 μg/ml	55.0±2.1*	37.6±1.5*	7.4±2.4*
Migrating				
	Not treated	45.9±0.7	36.9±3.1	17.2±1.2
	Doxorubicin 0.5 μg/ml	40.6±1.0*	51.6±2.9*	7.8±0.9*

Results are expressed as means±SD from 3-5 data.
* significantly different from the corresponding control group (not treated): ($p<0.05$).

Results

MMP activity and β1 integrin protein expression in OSCORT tumour cells exposed to ECM-gel. OSCORT cells grown on a plastic tissue culture dish did not constitutively have MMP activity, but a remarkable level of MMP-9 was detected upon exposure of these cells to ECM-gel (Figure 1A). Similarly, increased protein expression of β1 integrin was detected in OSCORT cells migrated into ECM-gel (Figure 1B). Increased activity of MMP and the higher expression of β1 integrin either indicate the response of OSCORT cells to the presence of ECM or represent the behaviour of the migrating OSCORT cells.

Effect of doxorubicin treatment on the proliferation and migration of OSCORT cells. The differential responses of non-migrating (remained in plastic cell culture dish) and migrating (isolated from the ECM-gel after 24 hours overlay) OSCORT cells to doxorubicin were dependent on the duration of the treatment time. Applying 4 hours exposure time, 0.01, 0.05 and 0.25 μg/ml doses of doxorubicin were ineffective (data not shown). Therefore, in the further experiments we used 0.5 and 5.0 μg/ml doses of doxorubicin for 4 hours, then the cells were overlaid by ECM-gel for 24 hours, and the cell numbers in the ECM-gel-migrating and in the remaining non-migrating compartments were determined. Using the 0.5 and 5.0 μg/ml doses of doxorubicin for 4 hours, a dose-dependent reduction was observed in total cell numbers (Figure 2A). In the control and in the short-term treatments, the majority of the cells were found in the ECM-gel (Figure 2B) even after administration of 5.0 μg/ml doxorubicin. The cell

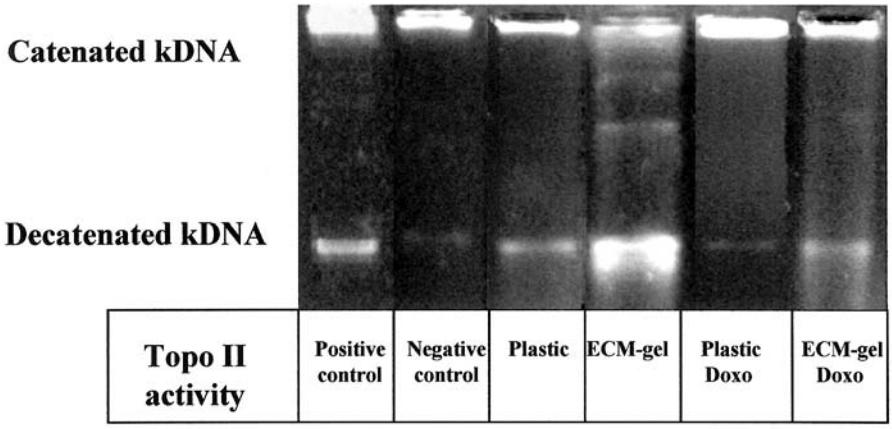


Figure 4. *Comparison of decatenation activity of topoisomerase II in OSCORT cells cultured on plastic surface or on ECM-gel. OSCORT cells were cultured with or without ECM-gel. Topoisomerase II activity was detected with decatenation in cell nuclear extracts of 2.5 x 10^4 OSCORT cells. Positive control: 2 units of calf thymus topoisomerase II; Negative control: kinetoplast DNA, without protein; Plastic: nuclear extracts from cells cultured on plastic tissue culture dish; ECM-gel: nuclear extracts from cells cultured on ECM-gel; Plastic Doxo: Nuclear extracts from cells cultured on plastic tissue culture dish and treated with 5 μg/ml doxorubicin, for 4 hours. ECM-gel Doxo: Nuclear extracts from cells cultured on ECM-gel and treated with 5 μg/ml doxorubicin for 4 hours. Topoisomerase II activity was analysed with densitometry.*

number reduction was less in the migrating cell population than in the non-migrating one.

Applying 24 hours exposure time, 0.01, 0.05 and 0.25 μg/ml doses of doxorubicin were already effective (Figure 2A), while administration of 0.5 μg/ml doxorubicin almost completely suppressed the whole cell population. Using the 0.01, 0.05 and 0.25 μg/ml doses of doxorubicin for 24 hours, a dose-dependent reduction was observed in total cell numbers (Figure 2A). On the other hand, the distribution between migrating and non-migrating cell populations changed, the majority of the cells remaining in the non-migrating compartment. The viability of the cells isolated from the ECM-gel after 4- or 24-hour treatment with doxorubicin was always 10% higher than the viability of the cells remaining in the monolayer. Our experimental data showed a dose-dependent antimigratory effect, but not increased cytotoxicity of long-term doxorubicin treatment on osteosarcoma cells.

Doxorubicin action on OSCORT cell population kinetics and topoisomerase II. A comparative flow cytometry study on the migrating and non-migrating OSCORT cells showed a higher frequency of S-phase cells in the migrating subpopulation. These data are in harmony with the increased ^{3}H-TdR incorporation into the DNA of these cells (data not shown). Doxorubicin treatment resulted in an even higher accumulation of S-phase cells (Table I).

Measurements on topoisomerase II deserve special interest for two reasons: a) it acts as the molecular target of doxorubicin, and b) dominancy of topoisomerase I, characteristic of the monolayer OSCORT cell culture, shifted to topoisomerase II upon migrating to the ECM-gel (manuscript submitted for publication by R. Harisi, J. Dudas, F. Timar, G. Pogany, M. Szendroi, A. Jeney, 2005). Figure 3 shows the remarkably higher expression and cleavage activity of topoisomerase II in the migrating OSCORT cells relative to those in the monolayer. The difference in the cleavage activity of topoisomerase II in OSCORT cells originated from the non-migrating and migrating compartment was apparent when etoposide was added to the reaction mixture (Figure 3B). Also worthy of note is the fact that doxorubicin similarly inhibited topoisomerase II activity both in the non-migrating and in the migrating OSCORT cell population (Figure 4). However, the immunocytochemical study showed that doxorubicin treatment prevented the elevation of topoisomerase II levels after exposure to ECM-gel (Figure 5).

Discussion

Although ECM alterations in malignant tumours were first observed a long time ago, their importance in tumour progression and in abnormal cellular regulation attracted attention only in the last decade (14, 28-31), and, most interestingly, in antitumour drug research only recently (16, 32). Since the microinvasive assay applied in this study involves migration of tumour cells from a monolayer culture to a 3-dimensional ECM-gel (28, 29), it offered an *in vitro* model for the seeding process in the metastatic cascade and had certain advantages over the chamber technique: a) proliferation and migration could be simultaneously assessed, and b) a large number of cells could be obtained for molecular

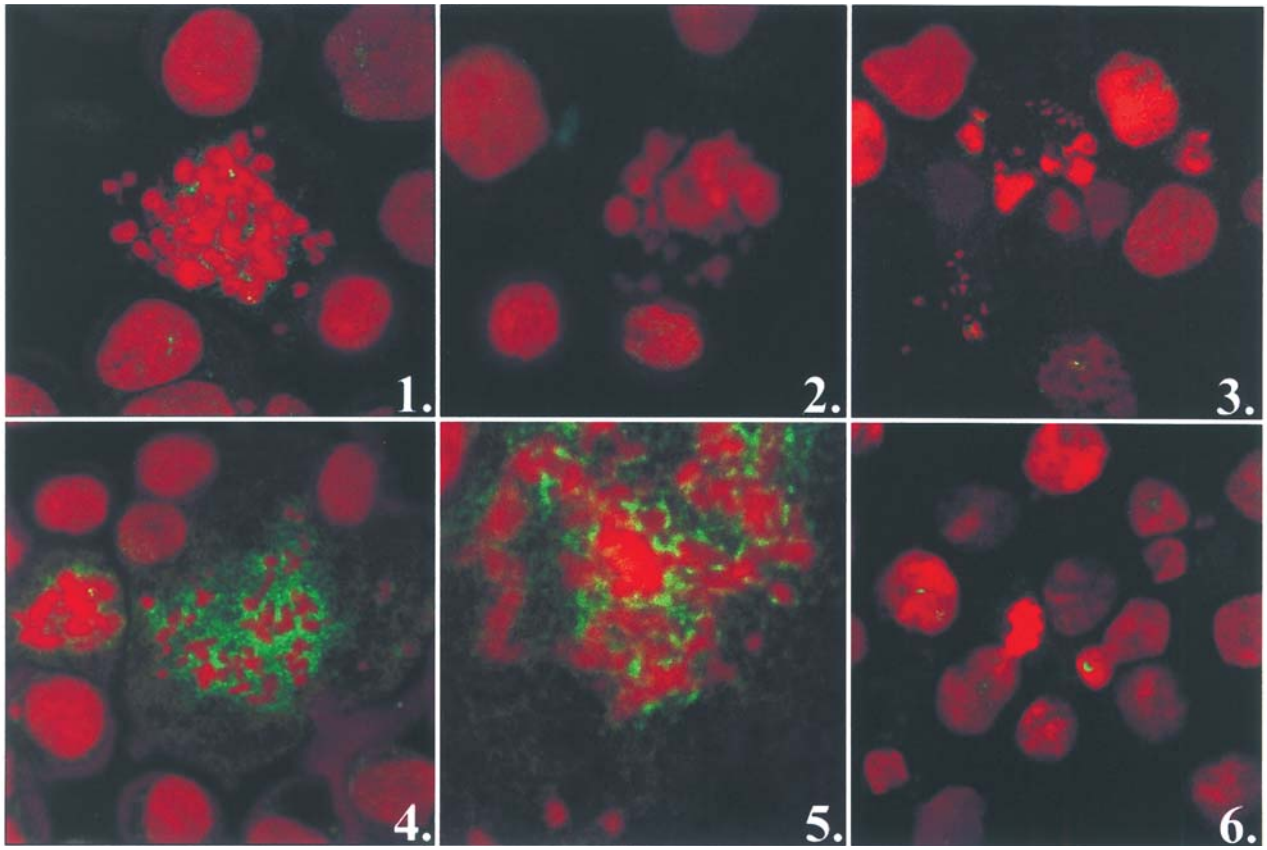


Figure 5. *Comparison of topoisomerase II immunocytochemical detection in OSCORT cells cultured on plastic surface or on ECM-gel. OSCORT cells were cultured with or without ECM-gel, treated with doxorubicin and antibody reaction against topoisomerase II was detected using confocal laser-scanning microscopy. Topoisomerase II is shown in green and cell nuclei were counterstained with propidium iodide (red). 1, 2: OSCORT cells were cultured on plastic for 72 hours; mitotic cells show very low topoisomerase II immunoreaction 3: OSCORT cells were cultured on plastic dish for 48 hours and treated in the last 4 hours with 5 μg/ml doxorubicin. After changing the medium the cells were cultured for a further 24 hours. The immunoreaction did not increase. 4, 5: OSCORT cells were plated on plastic dish and cultured for 48 hours, then ECM was overlaid and the 3-dimensional cultures were incubated for a further 24 hours. 6: OSCORT cells were plated on plastic dish, cultured for 48 hours and treated in the last 4 hours with 5 μg/ml doxorubicin, then ECM was overlaid and the 3-dimensional cultures were incubated for 24 hours. Mitotic cells show increased topoisomerase II immunoreaction in the presence of ECM-gel (1,2,3,4,6: 400x original magnification, 5: 1000x original magnification). Doxorubicin treated cells overlaid by ECM-gel show disturbed cell divisions and reduced topoisomerase II expression.*

studies. The 3-dimensional cultures bear more resemblance to an *in vivo* situation than do the monolayer cultures, therefore, the increased β1 integrin expression and the induction of MMP-9 activity may represent important events during the seeding stage of metastasis. MMP-9 was clearly induced by ECM-gel, while the cells in conventional monolayer culture did not have any constitutive expression of MMP-9.

The elevation of topoisomerase II concomitant to OSCORT cell migration is intriguing and seems to warrant further testing to determine whether it is a general feature in tumour invasion. The few available data on the relative activity of the two main types of topoisomerases indicate a higher activity of topoisomerase I in a proliferating tumour cell population. This is not inconsistent with the fact that topoisomerase II is mostly prevalent in late log and early plateau phase cultures and absent in non-cycling cells (33, 34). The elevation of topoisomerase II in cells migrated to ECM-gel may indicate increased transcription, DNA synthesis and repair functions, since this is one of the enzymes responsible for maintenance of the genome (35). We assume that the ECM-induced elevation of topoisomerase II may be an underlying event in the reprogramming of the gene expression, which is necessary for the invasive growth. As topoisomerase II showed higher levels of expression and activity in the migrating OSCORT cells relative to the proliferating monolayer, it was of interest to study whether the two subpopulations are affected to the same extent by doxorubicin as an inhibitor

of topoisomerase II. Doxorubicin caused similar reduction in the activity, but difference in the lowering of protein expression of topoisomerase II in the non-migrating and in the migrating cells (Figure 4 and Figure 5). Nevertheless, assuming the critical function of topoisomerase II in tumour invasion, it is conceivable that it could offer a molecular target for the selective antimigratory effect of doxorubicin applied at a low concentration for a long period. On the other hand, the antimigratory effect of doxorubicin was not accompanied by a decrease of cell viability.

In summary, new evidence has been provided to show the relevance of the ECM in the antitumour potency of doxorubicin. Using tumour cell migration into the ECM as a microinvasive assay, the antiproliferative and antimigratory effects of doxorubicin could be demonstrated. While the antiproliferative effect was more enhanced by short treatment at high concentrations, the antimigratory effect was more remarkable by long treatment at lower concentrations. In addition, changes in the biochemical features (high topoisomerase II activity) of the migrating OSCORT cells may be proportional to their altered chemosensitivity. Our experimental data might indicate different treatment schedules of doxorubicin in osteosarcoma with intentions to target tumour growth or invasion.

Acknowledgements

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