Thieme

Influence of Paclitaxel and Heparin on Vitality, Proliferation and Cytokine Production of Endometrial Cancer Cells

Auswirkung von Paclitaxel und Heparin auf die Vitalität, Proliferation und Produktion von Zytokinen bei Endometrium-karzinomzellen

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ABSTRACT

Background Cancer patients have a higher risk for thromboembolic events compared to healthy individuals and are often treated with heparins. A beneficial effect of heparins on tumor patients above and beyond the classic anticoagulation effect has been reported, leading to an increased focus on the use of heparins in anticancer treatment. In recent years, it has become apparent that microenvironments greatly affect tumor development and can be a major source of tumor-promoting factors. Cytokines play an important role in tumor microenvironments, inducing carcinogenesis and influencing tumor progression by promoting angiogenesis, metastatic potential and immunosuppression. The possible interaction of heparins and cytokines could also have an effect on cancer cells.

Methods This study investigated the effect of paclitaxel (PTX) combined with heparins on the vitality of endometrial cancer cells using viability and cytotoxicity assays. The study also examined whether treatment with paclitaxel and heparin influences cytokine secretion or expression.

Results Heparin treatment did not influence cell viability, and no influence of heparins in combination with paclitaxel was seen for the evaluated cancer cell lines HEC-1-A, KLE, RL 95-2 and AN3-CA compared to untreated cells. Secretion of the cytokines CCL5, CCL2 and IL-6 increased after paclitaxel treatment in several endometrial cancer cell lines, but no general effect on cytokine secretion was detected after heparin treatment. A significant decrease in CCL5 expression was only detected in KLE cells following treatment with heparin and paclitaxel, and an increase in the expression of CCL5 in RL 95-2 cells.

Conclusion Further in-depth studies are needed to investigate the functions of cytokines CCL2, CCL5 and IL-6 in endometrial cancer cells treated with paclitaxel. Although no general effect on cytokine secretion was detected following heparin treatment, a selective modulatory impact could exist.

ZUSAMMENFASSUNG

Hintergrund Verglichen mit gesunden Menschen haben Krebspatienten ein höheres Risiko für Thromboembolien und werden deshalb oft mit Heparin behandelt. Es wurde eine vorteilhafte Wirkung von Heparin auf Tumorpatienten festgestellt, die über die klassische Antikoagulation hinausgeht. Das hat die Aufmerksamkeit der Krebsforschung auf den Einsatz von Heparin in der Krebsbehandlung gelenkt. In den letzten Jahren wurde es klar, dass die Tumorentwicklung stark von der Mikroumgebung beeinflusst wird, die eine wichtige Quelle tumorfördernder Faktoren sein kann. Zytokine spielen eine

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wichtige Rolle für Tumormikroumgebungen: sie induzieren die Karzinogenese und können die Tumorprogression beeinflussen durch die Förderung der Angiogenese und des metastatischen Potenzials sowie der Immunsuppression. Die möglichen Interaktionen zwischen Heparin und Zytokine könnten auch Auswirkungen auf Tumorzellen haben.

Methoden Ziel dieser Studie war es, die Wirkung von Paclitaxel (PTX) in Kombination mit Heparin auf die Vitalität von Endometriumkarzinomzellen zu untersuchen mithilfe von Zellviabilitäts- und Zytotoxizitätsassays. Es wurde auch untersucht, ob eine Behandlung mit Paclitaxel und Heparin sich auf die Ausschüttung und Expression von Zytokinen auswirkt.

Ergebnisse Die Behandlung mit Heparin hatte keine Auswirkungen auf die Zellviabilität. Verglichen mit unbehandelten Zellen wurden für die untersuchten Krebszelllinien HEC-1-A, KLE, RL 95-2 und AN3-CA keine Auswirkungen von Heparin in Kombination mit Paclitaxel festgestellt. Die Ausschüttung der Zytokine CCL5, CCL2 und IL-6 verstärkte sich nach einer Behandlung mit Paclitaxel bei mehreren Zelllinien des Endometriumkarzinoms, aber es wurden keine generellen Auswirkungen auf die Ausschüttung von Zytokinen nach einer Behandlung mit Heparin festgestellt. Eine signifikante Verminderung der Expression von CCL5 nach einer Behandlung mit Heparin und Paclitaxel wurde nur bei KLE-Zellen gefunden, zusammen mit einer Steigerung der Expression von CCL5 in RL-95-2-Zellen.

Schlussfolgerung Weitere detaillierte Studien sind nötig, um die Funktionen der Zytokine CCL2, CCL5 und IL-6 in mit Paclitaxel behandelten Endometriumkarzinomzellen herauszuarbeiten. Obwohl kein genereller Effekt auf die Ausschüttung von Zytokinen nach einer Behandlung mit Heparin festgestellt werden konnte, ist eine selektive regulierende Wirkung nicht auszuschließen.

Introduction

Endometrial cancer is the most common gynecologic malignancy with a peak incidence between the ages of 55 and 65 years [1]. Around 142 000 women are affected by endometrial cancer worldwide every year [1]. Endometrial cancer is frequently diagnosed at an early stage as affected women often present with abnormal vaginal bleeding. In its early stages endometrial cancer can be treated surgically with intent to cure [2]. Adjuvant radiation therapy or adjuvant chemotherapy have been shown to improve the outcome of patients with high-risk endometrial cancer [3].

Around 13 % of all patients with endometrial cancer develop recurrent disease [2]. Different therapeutic modalities including radiotherapy and surgery and systemic therapies such as chemotherapy and hormone therapy are used to treat recurrent endometrial cancer [3]. Clinical trials evaluating chemotherapy regimens in patients with endometrial cancer include combinations of doxorubicin and cisplatin, cyclophosphamide or paclitaxel and carboplatin, most of them administered in a palliative setting [3]. These systemic treatment options are often accompanied by high toxicity.

Cancer patients have a generally higher risk for thromboembolic events compared to healthy individuals and are often treated with heparins [4]. Different studies have reported a synergistic effect of heparins above and beyond their standard anticoagulation effect in tumor patients; this has led to an increased focus on heparins in anticancer treatment. Lapierre et al. reported that treatment with heparin inhibited tumor growth of pancreatic adenocarcinoma in a mouse model [5]. Other studies found no antiproliferative effect but did report an inhibition of metastatic potential [6,7].

To develop new strategies for further therapeutic interventions, a detailed understanding of the molecular mechanisms in endometrial cancer is necessary. In recent years, it has become apparent that microenvironments greatly affect tumor development, because they are a major source of tumor-promoting factors for a huge range of tumors [8]. It is known that cytokines play an important role in tumor microenvironments: TNF-alpha and IL-6, for example, are proinflammatory cytokines which also induce carcinogenesis [9]. High serum levels of TNF-alpha, IL-6 and IL-8 are often detected in cancer patients, and very high IL-6 levels seem to be associated with a poor prognosis [10]. The CC-chemokine ligands CCL2 and CCL5 influence tumor progression by promoting angiogenesis, metastatic potential and immunosuppression [11,12].

Spratte et al. showed that heparins inhibited TNF-alpha signaling in endometrial stromal cells [13]. The possible interaction between heparins and cytokines could also affect cancer cells.

Doster and co-workers reported a selective modulatory impact of unfractionated heparin on the expression of cytokines (CXCL8, CCL2 and CCL5) in endometrial cancer cells [14].

Based on these findings, this study aimed to investigate the effects of treatment with paclitaxel and heparins on the vitality, proliferation and cytokine secretion of endometrial cancer cells.

Material and Methods

Endometrial cancer cell lines

Endometrial cancer cells were obtained from ATCC (Manassas, VA, USA). HEC-1-A were cultivated in McCoys medium (Biochrom AG, Berlin, Germany). KLE and RL 95-2 were cultivated in DMEM/F12 medium (Invitrogen, Karlsruhe, Germany) and AN3-CA ware cultivated in MEM Earle's medium (Invitrogen). All media contained 10% fetal bovine serum and 50 μg/mL gentamycin (Invitrogen) and cells were cultivated at 37 °C in a 5 % CO_2 humidified atmosphere.

Cell lines differ in their grading and pattern of metastatic spread; the cells of HEC-1 A and RL 95-2 are moderately differentiated and originate from the epithelial layer of the endometrium. KLE and AN3-CA are poorly differentiated cell lines of endometrial adenocarcinoma, with AN3-CA originating from lymph node metastasis [15].

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Cell lines were cultured 48 h prior to the different experiments. Different numbers of cells were used for all experiments as follows: 50 000 RL 95-2 cells, 30 000 HEC-1A cells, 15 000 KLE cells and 40 000 AN3CA cells.

Viability assay

Endometrial cancer cells of all four cell lines were treated for up to 144 h with 0.1 nM or 0.1 µM paclitaxel and/or 0.1 U/mL, 1 U/mL or 10 U/mL unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) (dalteparin/enoxaparin/reviparin/fondaparinux). All experiments were performed under normoxic (21% O_2) and hypoxic (1–5% O_2) conditions.

The relative number of viable endometrial cancer cells influenced by the indicated agents (paclitaxel and/or heparin) was measured at the end of each respective experiment (every 24 h between 0 h and 144 h) using the CellTiter-Blue assay (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. Fluorescence was recorded using the FLUOstar OPTIMA system (BMG Labtech, Offenburg, Germany). Untreated cells were used as controls. Excitation was measured at 560 nm and emission at 590 nm.

Cytotoxicity assay

Endometrial cancer cells of the four cell lines were treated for up to 144 h with 0.1 nM or 0.1 µM paclitaxel and/or 0.1 U/mL, 1 U/mL or 10 U/mL UFH. All experiments were performed under normoxic and under hypoxic conditions. The number of viable endometrial cancer cells influenced by the indicated agents (paclitaxel and/or heparin) was measured at the end of each respective experiment using the CytoTox-ONE homogeneous membrane integrity assay (Promega) in accordance with the manufacturer's instructions. The test was used to detect cytotoxicity when cytokine secretion was evaluated to differentiate between the active cytokine secretion of vital cells and passive cytokine release through cell death.

Enzyme-linked immunosorbent assay

Endometrial cancer cells were treated with different doses of paclitaxel (0.01 µM, 0.1 M or 1 µM) for 24 h and 72 h. At the end of each time period, enzyme-linked immunosorbent assays (ELISAs) were performed to detect cytokine secretion, using commercial ELISA kits to detect human IL-1β, IL-6, CCL2, CCL5 (R&D Systems, Wiesbaden, Germany) and TNF-alpha (eBioscience, Frankfurt, Germany) in accordance with the manufacturer's instructions. Absorbance values were measured with the Fluostar Optima system and normalized to the relative number of viable endometrial cancer cells.

Real-time reverse transcription polymerase chain reaction

To detect whether paclitaxel treatment alone influences the secretion of cytokines or cytokine expression, real-time reverse transcription polymerase chain reaction was performed. Endometrial cancer cells lines RL 95-2 and HEC-1A were treated with 0.01 µM, 0.1 µM or 1 µM paclitaxel for 72 hours. Untreated cells were used as controls.

Total ribonucleic acid (RNA) was isolated from endometrial cancer cells using peqGOLD Trifast (Peqlab) and reverse-tran-

▶ **Table 1** Sequences of primers used for PCR.

Forward/reverse primer sequence	
Human β-actin	5′-CCTGGCACCCAGCACAAT-3′ 5′-GCCGATCCACACGGAGTACT-3′
Human CCL5	5′-CTCGCTGTCATCCTCATTGCT-3′ 5′-TGTGGTGTCCGAGGAATATGG-3′

scribed using the high capacity cDNA archive kit (Applied Biosystems, Foster City, CA, USA).

Semi-quantitative real-time polymerase chain reaction (PCR) was performed to quantify the mRNA levels of CCL5 in relation to the housekeeping gene β-actin. cDNA samples were amplified with the Power Sybr Green PCR Master Mix (Applied Biosystems) and the respective forward and reverse primers. The primers (Invitrogen) were designed using Primer Express primer design software, version 2.0 (Applied Biosystems), with the resulting amplicons having an intron-overlapping sequence. The sequences of the primers used are summarized in ▶ **Table 1**.

PCR amplification was performed in duplicate in an ABI Prism 7300 sequence detector (Applied Biosystems) using a standard cycling program [16]. PCR products were analyzed by thermal dissociation to verify that a single specific PCR product had been amplified. Relative expression levels of CCL5 in relation to b-actin were determined using the mathematical model: ratio: ¼ 2DDCT [17].

Statistical analysis

Statistical analysis was done by one-way ANOVA, followed by Dunnett's and Bonferroni multiple comparison tests or unpaired Mann–Whitney t-tests, using GraphPad PRISM version 5.01 software (GraphPad Software Inc., La Jolla, CA, USA). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Viability assay

After treatment with UFH or LMWH no influence on the cell viability of the four different endometrial cancer cell lines could be demonstrated compared to untreated cells. As expected, the toxic PTX dose showed a decreasing signal in the CellTiter-Blue assay, which could not be influenced by combining PTX with heparins. Hypoxic conditions did not change the results demonstrated under normoxic conditions (data not shown).

Viability and cytotoxicity after treatment with paclitaxel

All four evaluated endometrial cancer cell lines showed a decrease in vital cells after treatment with paclitaxel compared to untreated cells. No significant change in membrane integrity was measured in the Cytotox-ONE assay after treatment with paclitaxel (data shown in ▶ **Fig. 1**).
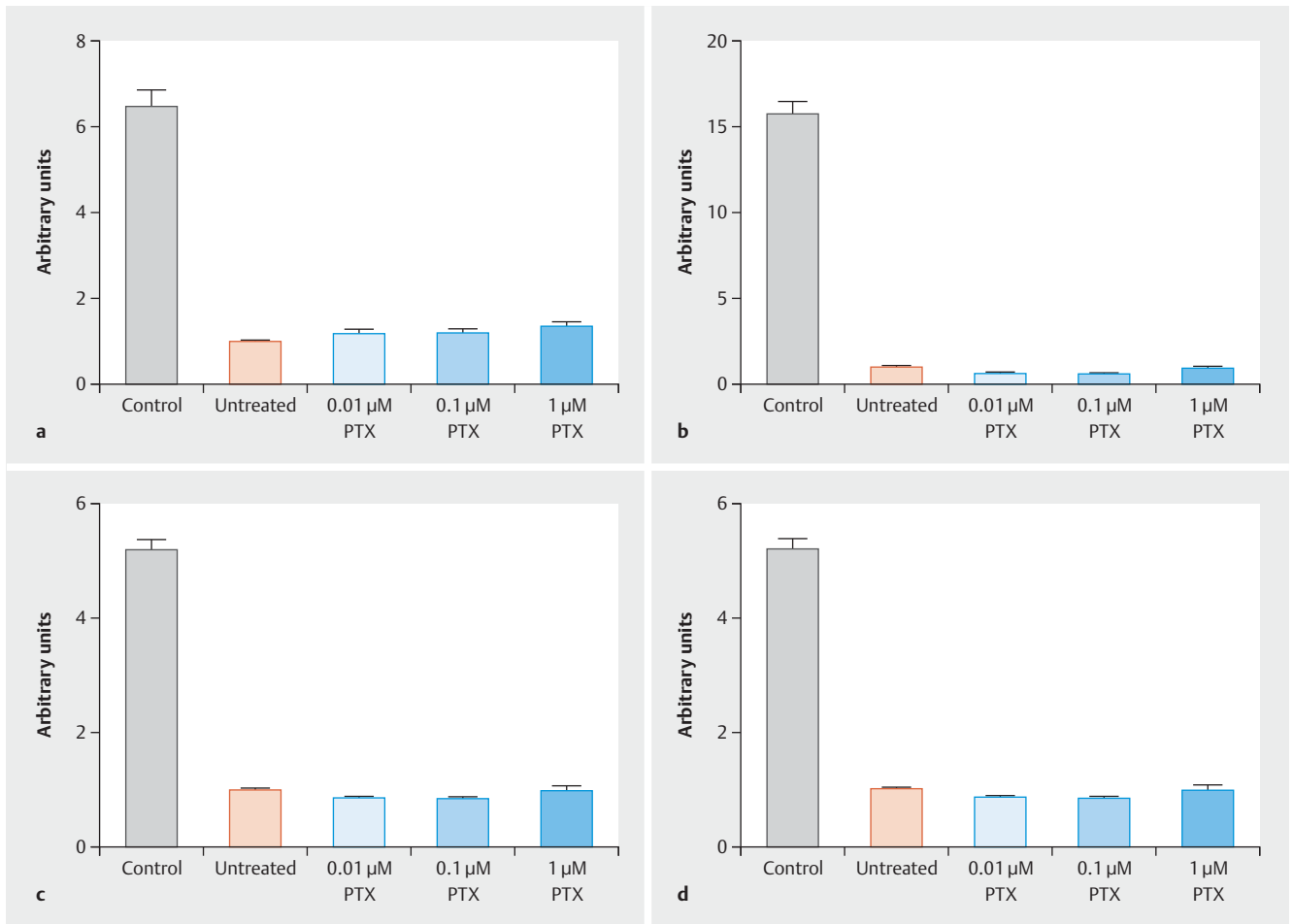
▶ **Fig. 1** Cytotoxicity assay for all four evaluated cell lines. No significant difference in membrane integrity was measured after treatment with paclitaxel (PTX). **a** AN3-CA, **b** HEC-1A, **c** KLE, **d** RL 95-2.

Basal cytokine secretion in untreated endometrial cancer cells

Basal secretion of IL-1β and TNF-alpha could not be detected in the four different cell lines due to values below the detection limit of the commercial ELISA kits used.

CCL5 secretion was shown in all four cell lines; the highest secretion was detected in HEC-1A cells (61 pg/mL/10 000 cells).

IL-6 was secreted by KLE, RL-95 and HEC-1A; the highest secretion was detected in KLE cells (18.6 pg/mL/10 000 cells). Only in AN3-CA cells was the secretion level too low to be detected.

CCL2 secretion was measured in RL-95, HEC-1A and KLE cells; in AN3-CA cells the secretion levels were again too low to be detected (data shown in ▶ **Table 2**).

Cytokine secretion after treatment with paclitaxel

CCL5 secretion increased 72 h after paclitaxel treatment in three of the examined cell lines. In HEC-1A and RL 95-2 cells, secretion changed significantly for all three doses of paclitaxel (0.01 M; 0.1 μM; 1 μM). In KLE cells secretion was reduced under paclitaxel treatment, but the results were only significant for 1 μM paclitaxel.

Secretion of IL-6 increased significantly after paclitaxel treatment in RL 95-2 cells. Treatment with 0.1 μM paclitaxel led to a 52-fold increase in secretion.

In HEC-1A cells the increase in secretion was only significant for 0.01 μM and 0.1 μM paclitaxel. In KLE cells, the secretion of IL-6 did not increase significantly and the secretion levels of IL-6 in AN3-CA cells were below the detection limit.

CCL2 secretion after paclitaxel treatment only showed a significant increase for HEC-1A cells (data shown in ▶ **Table 3**).

Influence of paclitaxel on cytokine expression of endometrial cancer cells

To determine whether paclitaxel treatment only affects the secretion of cytokines or whether it also affects cytokine expression, real-time reverse transcription polymerase chain reaction was performed.

In RL 95-2 cells, CCL5 mRNA expression increased 5.3-fold following treatment with 0.01 μM paclitaxel. The other paclitaxel doses used resulted in no significant changes in the mRNA expression of CCL5. In HEC-1A cells, the increase in CCL5 mRNA expression was even higher; treatment with 0.1 μM paclitaxel resulted in

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▶ **Table 2** Basal cytokine secretion in untreated endometrial cancer cells.

		CCL5	IL-6	CCL-2
RL 95-2	50 000 cells/well	14.2 pg/mL	2.15 pg/mL	310 p g/mL
	10 000 cells	2.8 pg/mL	0.43 pg/mL	62 pg/mL
HEC-1A	30 000 cells/well	183 pg/mL	3 pg/mL	95 pg/mL
	10 000 cells	61 pg/mL	1 pg/mL	32 pg/mL
KLE	15 000 cells/well	40.5 pg/mL	27.9 pg/mL	14 300 pg/mL
	10 000 cells	27 pg/mL	18.8 pg/mL	9 533 pg/mL
AN3-CA	40 000 cells/well	18 pg/mL	below detection limit	below detection limit
	10 000 cells	4.5 pg/mL		

▶ **Table 3** Induction of CCL5/IL-6/CCL2 after paclitaxel treatment (compared to untreated cells).

		0.01 µM PTX		0.1 µM PTX		1 µM PTX	
		Fold change SEM	Significance	Fold change SEM	Significance	Fold change SEM	Significance
CCL5	RL 95-2	17.7 ± 1.5	$p < 0.01$	19.5 ± 2.2	$p < 0.001$	10.6 ± 1.4	$p < 0.05$
	HEC-1A	4.5 ± 0.7	$p < 0.001$	3.7 ± 0.5	$p < 0.01$	2.7 ± 0.4	$p < 0.05$
	KLE	0.81 ± 0.08	Not significant	0.63 ± 0.04	Not significant	0.49 ± 0.11	$p < 0.05$
	AN3-CA	2.5 ± 0.6	Not significant	1.9 ± 0.4	Not significant	1.47 ± 0.12	Not significant
IL-6	RL 95-2	53.0 ± 6.0	$p < 0.001$	49.99 ± 1.93	$p < 0.001$	31.0 ± 4.0	$p < 0.01$
	HEC-1A	20.6 ± 2.6	$p < 0.001$	16.3 ± 1.9	$p < 0.01$	7.2 ± 0.8	Not significant
	KLE	1.83 ± 0.19	Not significant	2.9 ± 0.4	$p < 0.01$	2.3 ± 0.4	Not significant
	AN3-CA	Below the detection limit					
CCL2	RL 95-2	3.7 ± 0.6	$p < 0.05$	3.5 ± 0.5	$p < 0.05$	3.0 ± 0.4	Not significant
	HEC-1A	1.82 ± 0.08	$p < 0.001$	1.06 ± 0.10	Not significant	0.70 ± 0.04	Not significant
	KLE	0.85 ± 0.08	Not significant	0.97 ± 0.04	Not significant	0.63 ± 0.06	$p < 0.05$
	AN3-CA	Below detection limit					

a 58-fold increase in expression, and treatment with 0.1 µM paclitaxel increased expression 42-fold (data shown in ▶ **Fig. 2**).

Influence of paclitaxel combined with heparin on cytokine expression of endometrial cancer cells

In KLE cells, a statistically significant decrease in CCL5 expression was detected after treatment with paclitaxel and UFH.

In RL 95-2 cells, CCL5 expression increased after treatment with paclitaxel and UFH.

No significant differences were noted for the other examined cell lines (data not shown).

Discussion

Endometrial cancer is often detected in its early stages and can be treated and cured. But for patients with advanced or recurrent disease the therapeutic options are poor, and new strategies will have to be evaluated. Antitumor research has begun to focus on heparins because different studies have shown that cancer patients treated with heparins to prevent thromboembolic events

lived longer than cancer patients who did not receive anticoagulation treatment. In-vitro models and animal models showed an inhibition of tumor growth or metastatic potential following heparin treatment [5 – 7].

This study aimed to evaluate the effect of paclitaxel combined with heparins on the vitality of endometrial cancer cells. The study also investigated whether paclitaxel and heparin treatment affects cytokine secretion or expression. Cytokines play a pivotal role in tumor microenvironments and influence the activation and migration of immune cells [8]. The chemokines CCL2 and CCL5 influence tumor progression by promoting angiogenesis, metastatic potential and immunosuppression [11, 12]. Higher serum levels of IL-6 have been found in breast cancer patients compared to healthy individuals, and higher serum levels correlated strongly with tumor stage and lymph node metastasis [18]. Ma et al. reasoned that serum levels might help to identify patients with a poor prognosis [18]. Another study showed that CCL2 and CCL5 are highly expressed by breast cancer cells compared to regular breast epithelial cells, suggesting that both cytokines play a role in the development and progression of breast cancer. It could
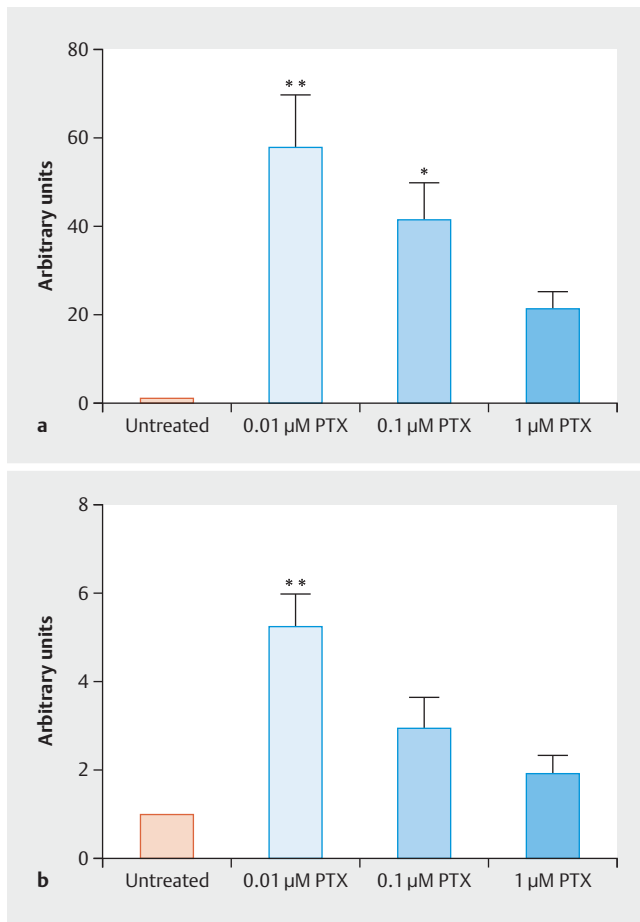
▶ **Fig. 2** Results of real-time PCR to evaluate CCL5 expression after treatment of **a** HEC-1A cells and **b** RL 95-2 cells with different doses of paclitaxel (0.01 µM; 0.1 µM and 1 µM).

be demonstrated that both chemokines promote a pro-malignant phenotype of cancer cells. These findings suggest that CCL2 and CCL5 could be potential therapeutic targets in breast cancer treatment [19, 20].

In our study, treatment with paclitaxel was found to affect the expression and secretion of CCL2, CCL5 and IL-6. An increase in the secretion of CCL2, CCL5 and IL-6 was detected; this could promote angiogenesis and metastatic potential but could also be a sign of the migration of immune cells into the tumor microenvironment. But the results and changes in secretion or expression varied considerably between the different cell lines, and further evaluations into the mechanistic processes and regulations will be needed to determine the purposes of the different cytokines in endometrial cancer. Tumor microenvironments are very complex; it is known that cytokines play a pivotal role [8], but findings of other studies vary considerably and further studies will be necessary to identify the concrete functions of the above-mentioned cytokines in endometrial cancer and other entities.

No overall effect on cytokine secretion was seen after heparin treatment (UFH or LMWH). A significant decrease in CCL5 secretion was only detected in KLE cells. The combination of UFH and paclitaxel had a synergistic effect on the increase of CCL5 secre-

tion in RL-95 cells. This indicates that heparins seem to have a selective impact on cytokine secretion and may not generally influence cytokine secretion. This finding makes heparins very interesting and could be used to affect tumor microenvironments in specific ways by influencing the expression of inflammatory mediators in cancer cells. It has been shown, for example, that in breast cancer cells unfractionated heparin and LMWH had an inhibitory effect on the production of different cytokines (CXCL9 and CXCL10) [21]. The authors reported that the observed effects were independent of the anticoagulation effects but depended on the molecular size and negative charge of the different polyanions [21]. Similar findings were also described by Fluhr et al. for endometrial stroma cells [22]. The effects of heparins also seemed to be independent of their anticoagulation effects but depend on molecular size and negative charge. This could be an interesting starting point for further studies of heparins in the setting of endometrial cancer.

No antiproliferative effect of heparins was detected in our study and there was no increase in the antimitotic effect of paclitaxel when it was combined with heparin. Heparins do not appear to influence cytotoxic treatment negatively either.

Further studies are needed to investigate the function of cytokines CCL2, CCL5 and IL-6 in endometrial cancer cells treated with paclitaxel. Studies into mechanistic processes and regulations will be necessary to determine the purposes of the different cytokines in the setting of endometrial cancer.

Although no general effect of heparin treatment on cytokine secretion was detected, a selective modulatory impact could exist and further evaluations are needed to analyze the impact of heparins on tumor microenvironments.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5–29

[2] van Wijk FH, van der Burg ME, Burger CW et al. Management of surgical stage III and IV endometrioid endometrial carcinoma: an overview. Int J Gynecol Cancer 2009; 19: 431–446

[3] Ray M, Fleming G. Management of advanced-stage and recurrent endometrial cancer. Semin Oncol 2009; 36: 145–154

[4] Levitan N, Dowlati A, Remick SC et al. Rates of initial and recurrent thromboembolic disease among patients with malignancy versus those without malignancy. Risk analysis using Medicare claims data. Medicine (Baltimore) 1999; 78: 285–291

[5] Lapierre F, Holme K, Lam L et al. Chemical modifications of heparin that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, anti-tumor and anti-metastatic properties. Glycobiology 1996; 6: 355–366

[6] Mousa SA, Petersen LJ. Anti-cancer properties of low-molecular-weight heparin: preclinical evidence. Thromb Haemost 2009; 102: 258–267

[7] Niers TMH, Klerk CPW, DiNisio M et al. Mechanisms of heparin induced anti-cancer activity in experimental cancer models. Crit Rev Oncol Hematol 2007; 61: 195–207

[8] Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci 2012; 125 (Pt 23): 5591–5596

Dinkic C et al. Influence of Paclitaxel … Geburtsh Frauenheilk 2017; 77: 1104–1110

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[9] Landskron G, De la Fuente M, Thuwajit P et al. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res 2014; 2014: 149185

[10] Lippitz BE. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol 2013; 14: e218–e228

[11] Yadav A, Saini V, Arora S. MCP-1: chemoattractant with a role beyond immunity: a review. Clin Chim Acta 2010; 411: 1570–1579

[12] Aldinucci D, Colombatti A. The inflammatory chemokine CCL5 and cancer progression. Mediators Inflamm 2014; 2014: 292376

[13] Spratte J, Meyer zu Schwabedissen H, Endlich N et al. Heparin inhibits TNF-α signaling in human endometrial stromal cells by interaction with NF-κB. Mol Hum Reprod 2013; 19: 227–236

[14] Doster A, Schwarzig U, Zygmunt M et al. Unfractionated heparin selectively modulates the expression of CXCL8, CCL2 and CCL5 in endometrial carcinoma cells. Anticancer Res 2016; 36: 1535–1544

[15] Dawe CJ, Banfield WG, Morgan WD et al. Growth in continuous culture, and in hamsters, of cells from a neoplasm associated with acanthosis nigricans. J Natl Cancer Inst 1964; 33: 441–456

[16] Fluhr H, Bischof-Islami D, Krenzer S et al. Human chorionic gonadotropin stimulates matrix metalloproteinases-2 and -9 in cytotrophoblastic cells and decreases tissue inhibitor of metalloproteinases-1, -2, and -3 in decidualized endometrial stromal cells. Fertil Steril 2008; 90: 1390–1395

[17] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25: 402–408

[18] Ma Y, Ren Y, Dai Z-J et al. IL-6, IL-8 and TNF-α levels correlate with disease stage in breast cancer patients. Adv Clin Exp Med 2017; 26: 421–426

[19] Soria G, Yaal-Hahoshen N, Azenshtein E et al. Concomitant expression of the chemokines RANTES and MCP-1 in human breast cancer: a basis for tumor-promoting interactions. Cytokine 2008; 44: 191–200

[20] Soria G, Ben-Baruch A. The inflammatory chemokines CCL2 and CCL5 in breast cancer. Cancer Lett 2008; 267: 271–285

[21] Fluhr H, Seitz T, Zygmunt M. Heparins modulate the IFN-γ-induced production of chemokines in human breast cancer cells. Breast Cancer Res Treat 2013; 137: 109–118

[22] Fluhr H, Spratte J, Heidrich S et al. The molecular charge and size of heparins determine their impact on the decidualization of human endometrial stromal cells. Mol Hum Reprod 2011; 17: 354–359

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Dinkic C et al. Influence of Paclitaxel… Geburtsh Frauenheilk 2017; 77: 1104–1110