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Dubious effects of methadone as an "anticancer" drug on ovarian cancer cell-lines and patient-derived tumor-spheroids

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HIGHLIGHTS

- The use of methadone as an anti-cancer drug is controversially discussed in Europe.
- Its effects in OC are completely unexplored.
- We could not find any benefit as a chemosensitizing anticancer drug in OC patients.
- Potentially dangerous and harmful effects of methadone have been identified.

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ABSTRACT

Background. The opioid agonist D,L-methadone exerts analgesic effects via the mu opioid receptor, encoded by *OPRM1* and therefore plays a role in chronic pain management. In preclinical tumor-models D,L-methadone shows apoptotic and chemo-sensitizing effects and was therefore hyped as an off-label "anticancer" drug without substantiation from clinical trials. Its effects in ovarian cancer (OC) are completely unexplored.

Methods. We analyzed *OPRM1*-mRNA expression in six cisplatin-sensitive, two cisplatin-resistant OC cell-lines, 170 OC tissue samples and 12 non-neoplastic control tissues. Pro-angiogenetic, cytotoxic and apoptotic effects of D,L-methadone were evaluated in OC cell-lines and four patient-derived tumor-spheroid models.

Results. OPRM1 was transcriptionally expressed in 69% of OC-tissues and in three of eight OC cell-lines. D,L-methadone exposure significantly reduced cell-viability in five OC cell-lines irrespective of *OPRM1* expression. D,L-methadone, applied alone or combined with cisplatin, showed no significant effects on apoptosis or VEGF secretion in cell-lines. Notably, in two of the four spheroid models, treatment with D,L-methadone significantly enhanced cell growth (by up to 121%), especially after long-term exposure. This is consistent with the observed attenuation of the inhibitory effects of cisplatin in three spheroid models when adding D,L-methadone. The effect of methadone treatment on VEGF secretion in tumor-spheroids was inconclusive.

Conclusions. Our study demonstrates that certain OC samples express *OPRM1*, which, however, is not a prerequisite for D,L-methadone function. As such, D,L-methadone may exert also detrimental effects by stimulating the growth of certain OC-cells and abrogating cisplatin's therapeutic effect.

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1. Introduction

Ovarian cancer (OC) is the fourth most common cause of cancer death and the leading cause of mortality among gynecological malignancies in the Western world [1]. The highly fatal nature of OC is due to a diagnostic delay stemming from the absence of symptoms in the majority of women with early stages of the disease, the lack of diagnostically useful biomarkers and the therapeutic limitations in therapies due to the development of drug resistance during treatment. In particular, patients with platinum-resistant/refractory OC face a very poor prognosis.

Although the use of the opioid-receptor agonist methadone as an anti-cancer drug is deemed controversial, this drug is gaining popularity in Europe as an "off-label" therapy despite insufficient research to support its benefits [2]. Methadone, introduced in the 1960's as a synthetic long-acting oral opiate, is a unique mu-opioid-receptor (MOR) agonist commonly used in substitution therapy for heroin dependence [3]. It is also used as a long-acting analgesic, particularly for neuropathic pain syndromes [4–6].

The potential role of methadone in treating cancer is supported by studies of glioblastoma and leukemia cell cultures, in which chemotherapy appears to be more effective at inducing cell death when supplemented with the opioid D,L-methadone [7,8]. By activating the opioid-receptor and downregulating cyclic adenosine monophosphate (cAMP), D,L-methadone can activate apoptotic signaling pathways and block anti-apoptotic molecules that may lead to chemotherapy resistance [9].

In addition to possible apoptotic effects, opiates may act via vascular endothelial growth factor (VEGF) receptors to stimulate VEGF-secretion, and secondarily angiogenesis, in certain cells [10]. This is of great importance since angiogenesis is known to support tumor growth. Whether opiates may exert comparable effects in OC-cells has not been studied to date.

Although endogenous opioids have been long recognized as important modulators within the central nervous system, opioid receptors have also been identified on human ovarian granulosa cells, oocytes and in the human endometrium [10–12]. However, the expression of opioid receptors in OC has not been studied so far.

The aims of this study were (i) to analyze the expression of the MOR OPRM1, the main binding site of methadone, in OC cell-lines and tumor tissues from OC-patients and (ii) to evaluate cytotoxic, apoptotic and angiogenic effects of methadone on OC cell-lines in two-dimensional (2D) cell-cultures and in patient-derived tumor-spheroids in three-dimensional (3D) systems.

2. Materials and methods

2.1. Drugs and reagents

D,L-methadone hydrochloride (D,L-methadone) was obtained from the local hospital pharmacy (Medical University Innsbruck), cisplatin, from Sandoz Biopharmaceuticals and Oncology Injectables (Unterach, Austria). Prior to each experiment, these substances were freshly dissolved in sterile distilled water to ensure the constant quality of the preparations.

2.2. OC cell-lines

A2780 and A2780Cis were purchased from Sigma-Aldrich (Vienna, Austria), HTB77 (=SKOV-3) and OVCAR3 (=HTB-161) from ATCC. SKOV6 cells were kindly provided by Lloyd Old (Sloan-Kettering Institute for Cancer Research, New York, USA). This cell-line is described incorrectly as problematic based on a report by Korch et al. in which the STR-profile of their "SKOV6" cell-line matched the profile of the "HTB-131" cervical cancer cell-line [13]. But the original SKOV6 cell line, which we received in 1991, has a completely different STR-profile as published by Beaufort et al. in 2014 [14]. The HOC7 was a gift from Thomas Grunt (Medical University Vienna, Vienna, Austria) and Olli Carpén (University of Turku, Turku, Finland) kindly provided M019i, a primary chemo-sensitive cell-line and its cisplatin-resistant derivative cell-line [15]. Amplification of 15 STR-loci and the gender-specific locus Amelogenin was carried out in the Institute of Legal Medicine, Medical University of Innsbruck, to authenticate these cell-lines as described recently [16].

2.3. 2D cell culture

The OC cell-lines were cultured in appropriate medium (i.e., RPMI or Dulbecco's modified Eagle's medium; PAA Laboratories; GE Healthcare, Munich, Germany) supplemented with 10% fetal bovine serum (Biochrom, Cambridge, U.K.), 2 mM L-glutamine, and 1× penicillin/streptomycin (Gibco; Life Technologies, Thermo Fisher Scientific, Waltham, MA). Cells were treated with 10 μg/ml methadone and cisplatin (5 μM: HOC7, HTB77; 10 μM: SKOV6; 1 μM: OVCAR3, A2780, A2780Cis, M019i, M019iCis).

2.4. Flow cytometric assay for determination of cell surface OPRM1

The analysis was performed on a FACS Calibur cytometer (BD Biosciences, Franklin Lakes, USA) by indirect staining using an OPRM1 goat polyclonal antibody (sc-7489, Santa Cruz Biotechnology, Dallas, USA) and donkey anti goat secondary antibody coupled to PE (705–116-147; Jackson ImmunoResearch Laboratories, West Grove, USA) [10].

2.5. Cell proliferation assays

Cell proliferation was determined with the MTT assay (Sigma Aldrich, Vienna, Austria) as recently described [17].

2.6. Flow cytometry for apoptosis analysis

Cell death was determined by staining the cells with propidium iodide/Triton-X100 (Sigma-Aldrich) and forward/sideward-scatter-analysis using a Beckman Coulter Cytomics FC-500 as described previously [18].

2.7. 3D cell culture – Tumor-spheroids

Tumor-spheroids were obtained during surgery from (i) ascites specimens of two patients with tubo-ovarian high-grade serous carcinoma (HGSC; patients were 78 and 61 years old) and (ii) tumor specimens from two other HGSC-patients (patients were 61 and 54 years old). None of the patients received methadone therapy as part of their clinical treatment. The study was reviewed and approved by the Ethics Committee of the Medical University of Innsbruck (reference no. 1054/2019) and conducted in accordance with the Declaration of Helsinki. Samples were cultured in a custom-constructed, fully 3D-printed, micro-processor-controlled bioreactor system that allows the simultaneous cultivation of up to 48 wells at controlled conditions in a cell culture incubator (Ausserlechner et al., manuscript submitted). After 72 h, the cells were treated with 10 μg/ml methadone and 10 μM cisplatin. Media and drugs were exchanged every 72 h. Size and morphology was monitored regularly by live-cell microscopy and cell death was visualized by propidium iodide staining. ATP-amount was analyzed as a marker for cell viability measured by CellTiter-Glo-3D-cell-viability-assay (Promega, Madison, USA).

2.8. VEGF-immunoassay

A human enzyme linked immunosorbent assay (ELISA) kit was used to determine VEGF-concentrations (Quantikine VEGF-ELISA; R&D

Systems, Minneapolis, MN, USA) as recently described [10]. The results were normalized to the cell numbers.

2.9. OPRM1-mRNA expression analysis in OC cell-lines and OC-tissues

Fresh frozen ovarian tissue samples were available from 170 patients with OC and non-neoplastic tubal tissues from 12 patients with benign diseases. They were obtained at primary debulking surgery (patients were 24 to 90 years old; median age at diagnosis was 61.3 years). Non-neoplastic tubal tissues from 12 patients were obtained by elective salpingo-oophorectomy for benign conditions (patients were 30 to 73 years old, median age: 50.4 years). Samples were collected and processed at the Department of Obstetrics and Gynecology of the Medical University of Innsbruck, Austria between 1989 and 2015 as described recently [19]. The collection of eligible samples during this time-period was based on a purposive sampling strategy. Only tissues with a tumor content of >90% were included.

The study was reviewed and approved by the Ethics Committee of the Medical University of Innsbruck (reference no. 1054/2019) and conducted in accordance with the Declaration of Helsinki. Total cellular RNA-extraction, reverse-transcription and quantitative real-time PCR (qPCR) were performed as previously described [20]. The qPCR was conducted in duplicates. Primers and probe for *OPRM1* were purchased from Applied Biosystems (Foster City, CA, USA, Applied Biosystems Assay ID: Hs01053957_m1). Data normalization was carried out against the endogenous RNA-control TATA box-binding protein (*TBP*) mRNA expression and expressed in arbitrary units.

2.10. OPRM1 immunohistochemistry in OC-tissues

Immunohistochemistry (IHC) was performed using an automated immunostainer (BenchMark ULTRA, Ventana Medical Systems, Tucson, AZ, USA). In short, formalin-fixed, paraffin-embedded (FFPE) tissue sections from 15 patients were prepared with Cell conditioning solution (CC1; Roche Diagnostics, Rotkreuz, Switzerland) for antigen retrieval. Anti-OPRM1 antibody (Sigma-Aldrich, HPA014509, St. Louis, MO, 1:20) was incubated for 32 min at 37 °C and for visualization the Ultra View DAB Detection Kit (Ventana Medical Systems, Oro Valley, AZ, USA) was used as recommended. Slides were counterstained with haematoxylin and bluing reagent. Images were acquired with a Zeiss AxioCam (Oberkochen, Germany). Testis tissue was used as a positive control.

2.11. Statistics

Statistical significance of differences between controls and treated cells were assessed using parametric and non-parametric approaches depending on the data distribution (e.g. Student's *t*-test or with Mann–Whitney *U* test or Kruskal-Wallis test). All statistical analyses were performed using SPSS v.24 (SPSS, Chicago, IL).

3. Results

3.1. OPRM1-expression in OC cell-lines

The *OPRM1*-mRNA and protein expression of six cisplatin-sensitive and two cisplatin-resistant OC cell-lines was analyzed by qPCR and FACS analysis. In Table 1 the expression status is described. In HOC7, SKOV6, HTB77 and M019i cells no expression at the mRNA and the protein level was observed. A very weak *OPRM1*-mRNA expression was detected in OVCAR3 and cisplatin-sensitive and resistant A2780 cells. At the protein level, the expression was observed only in OVCAR3.

Table 1
OPRM1-mRNA and protein expression in OC cell-lines.

Ovarian cancer cell line	OPRM1 expression	
	mRNA	protein
HOC7	not detectable	not detectable
SKOV6	not detectable	not detectable
HTB77	not detectable	not detectable
OVCAR3	positive	positive
A2780	positive	not detectable
A2780 Cisplatin resistant	positive	not detectable
M019i	not detectable	not detectable
M019i Cisplatin resistant	not detectable	not detectable

3.2. OPRM1 expression in ovarian tissues

Using qPCR, *OPRM1*-mRNA-expression was detectable in 69% (117/170) of OC samples and in 42% (5/12) of non-neoplastic fallopian tubes. However, *OPRM1* mRNA expression in these samples was very low: median expression in *OPRM1*-positive samples was 447-fold lower in the 117 ovarian cancer samples and 705-fold lower in control tissues compared to the reference gene. No differences in *OPRM1*-mRNA expression levels were observed between the diverse histologies, and no differences were found in HGSC compared to non-neoplastic fallopian tubes.

In association analyses, higher *OPRM1*-mRNA expression was seen in tumors from patients with no residual disease ($p = 0.022$) (Table 2).

To elucidate the intra-tumor OPRM1 protein distribution, we performed immunohistochemical analyses on 15 OC tissues with higher levels of *OPRM1* mRNA expression, but no protein expression could be detected in these tissues (the control testis tissue was highly positive (data not shown).

3.3. Effects of D,L-methadone alone or in combination with cisplatin on proliferation, apoptosis and VEGF-secretion of 2D cultured OC cell-lines

We treated HOC7, SKOV6, HTB77, OVCAR3, A2780, A2780Cis, M019i and M019iCis OC cell-lines with D,L-methadone and cisplatin and the combination of both for 72 h. The treatment with D,L-methadone alone reduced cell-viability in SKOV6 cells by 24% ($p = 0.004$), in OVCAR3 by 16% ($p = 0.004$), in A2780 cells by 8% ($p < 0.001$), in A2780Cis by 11% ($p = 0.001$) and in M019i by 6% ($p = 0.002$) in comparison to untreated controls (Fig. 1). No significant effect was observed

Table 2
Association of OPRM1 mRNA-expression with clinicopathological features in 170 ovarian cancer patients.

Variable	Number (%)	Median *OPRM1* mRNA expression values (arbitrary units)	*P*-value
Age			
<61.3 years (median-age)	85 (50%)	0.00076	0.069
>61.3 years (median-age)	85 (50%)	0.00108	
FIGO stage			
I/II	42 (25%)	0.00103	0.647
III/IV	128 (75%)	0.00097	
Tumor grade			
1	13 (8%)	0.00081	0.703
2	77 (45%)	0.00107	
3	80 (47%)	0.00098	
Residual disease			
macroscopically tumor-free	86 (51%)	0.00141	**0.022**
any tumor residual	78 (46%)	0.00052	
n.a.	6 (4%)	–	
Histology			
HGSC	136 (65%)	0.00099	0.148
LGSC	15 (6%)	0.00081	
endometrioid	9 (23%)	0.00176	
clear cell	10 (5%)	0.00038	

n.a. not available; Bold values denote statistical significance at the $p < 0.05$ level.

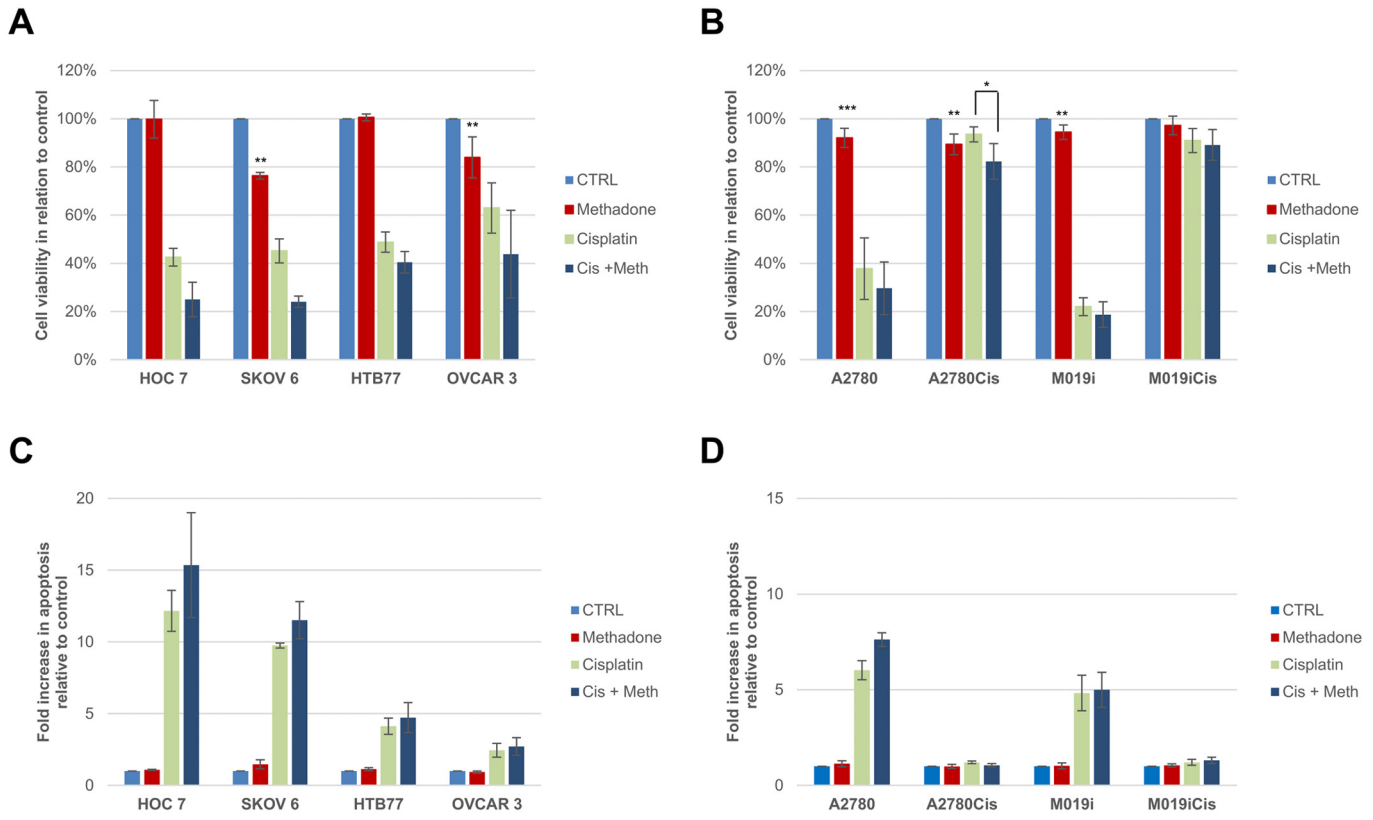

Fig. 1. Effects of D,L-methadone treatment on cell-viability and apoptosis in OC cell-lines. Cell-viability analysis in (A) HOC7, SKOV6, HTB77, OVCAR3 and (B) A2780, A2780Cis, M019i and M019iCis OC cell-lines. Apoptosis analysis in (C) HOC7, SKOV6, HTB77, OVCAR3 and (D) A2780 and A2780Cis, M019i and M019iCis OC cell-lines. Cells were treated for 72 h with D,L-methadone (10 μg/ml) and 5 μM cisplatin: HOC7, HTB77; 10 μM cisplatin: SKOV6; 1 μM cisplatin: OVCAR3, A2780, A2780Cis, M019i and M019iCis; or with a combination of both components. Cell proliferation was estimated by MTT assay. The means in relation to the untreated control cells are shown (± standard deviations of at least three independent experiments). Statistical significance is indicated by asterisks representing *p*-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

in HOC7, HTB77 and M019iCis (Fig. 1). Since D,L-methadone is discussed as being a sensitizer for chemotherapy, we tested its effects on the cisplatin treatment. The combination of D,L-methadone and cisplatin showed no supportive effects in these cell-lines in comparison to the monotherapy with cisplatin (Fig. 1A). Only in A2780Cis the combined treatment had a statistically significant stronger inhibitory effect on cell-viability than monotherapy, a 11% difference in comparison to cisplatin alone was observed ($p = 0.029$; Fig. 1B).

In apoptosis analysis, neither the treatment with methadone alone in comparison to untreated cells nor the combined treatment in comparison to cisplatin as a single agent revealed statistically significant effects (Fig. 1C, D). In addition, no effects of methadone on VEGF-secretion were observed (Fig. 2A, B).

3.4. Effects of D,L-methadone alone and in combination with cisplatin on proliferation and VEGF-secretion in 3D cultured patient-derived tumor-spheroids

Tumor-spheroids isolated from the ascites of two OC-patients (patient 1, patient 2) and minced tumor specimens from two other OC-patients (patient 3, patient 4) were cultivated in a custom-constructed bioreactor system.

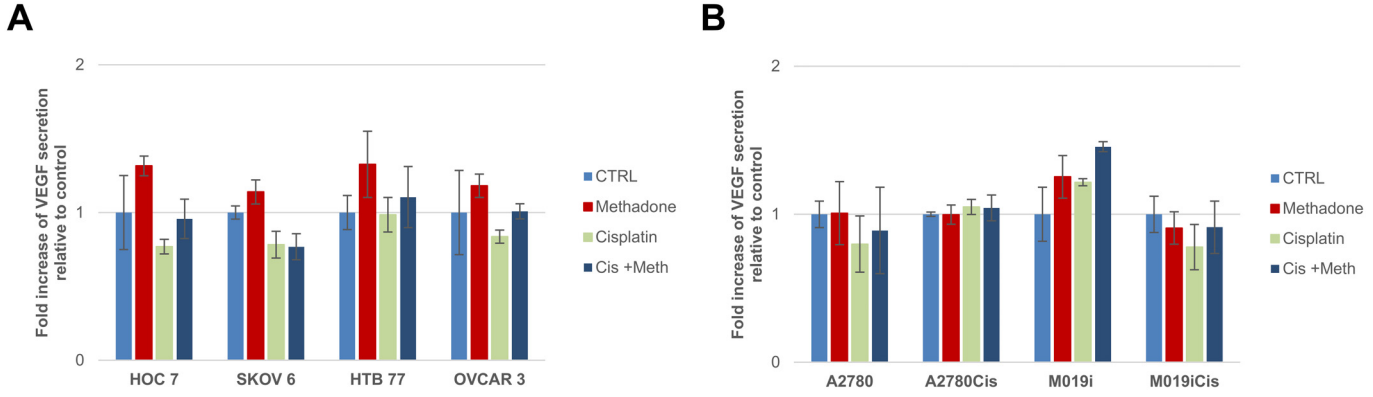

Fig. 2. Effects of D,L-methadone treatment on VEGF-secretion in OC cell-lines. VEGF-secretion analysis in (A) HOC7, SKOV6, HTB77, OVCAR3 OC cell-lines which were treated with 1 μM cisplatin, 10 μg/ml D,L-methadone alone or with a combination of both components for 24 h and in (B) A2780 and A2780Cis, M019i and M019iCis OC cell-lines which were treated with 1 μM cisplatin, 10 μg/ml D,L-methadone alone or with a combination of both components for 24 h. VEGF-secretion was measured by means of an ELISA. Results from three independent experiments are shown (± standard deviations). Statistical significance is indicated by asterisks representing p-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

In patient 1 the treatment with D,L-methadone alone led to a 21% and 18% reduction of the cellular viability of the tumor-spheroids in comparison to the untreated spheroids following 3 and 6 days of cultivation, respectively ($p = 0.002$; Fig. 3A). Interestingly, however, an increase in cell viability of 42% and 115% was observed with long-term treatment after 16 and 24 days, respectively. ($p = 0.002$). In OC spheroids from patient 2, an increase in cell-viability upon D,L-methadone treatment was observed throughout the cultivation period (day 3: 16%; day 6: 40%; day 18: 121%; day 24: 39%; $p = 0.002$; Fig. 3B). In spheroids derived from minced tumor specimens from two additional patients no significant effect on cell viability was revealed (Fig. 3C, D).

Of particular note, in spheroids from patient 1 and 2, the addition of D,L-methadone to cisplatin counteracted the cisplatin effect and resulted in up to 23% and 41% weaker reduction in cellular viability compared to cisplatin monotherapy, respectively ($p = 0.002$ for each; Fig. 3A, B). Likewise, in patient 4 we also observed this platinum antagonistic effect on viability (17%), but here with a time delay that occurred after 15 days ($p = 0.002$; Fig. 3D).

Only in patient 3, did we observe an 8% increase in cytotoxic platinum effect with combined treatment after 6 days ($p = 0.015$), but this disappeared after 12 days (Fig. 3C).

D,L-methadone induced VEGF secretion in tumor spheroids from patient 1 ($p = 0.037$; Fig. 4A). The combined treatment initially showed no additive effects on VEGF-secretion, but after 12 days a 1.9-fold increase of VEGF-secretion was observed compared to the cisplatin-monotherapy ($p = 0.034$; Fig. 4A). Already after 6 days, patient 3 showed a 2.9-fold increase in VEGF secretion after combination treatment, but this disappeared after 12 days (Fig. 4C).

In contrast, treatment with D,L-methadone in the tumor spheroids of patient 2 and patient 4 resulted in decreased VEGF secretion throughout the cultivation period (each $p = 0.037$, Fig. 4B, Fig. 4D). After combined treatment, we observed reduced VEGF secretion in these patients (each $p = 0.05$, Fig. 4B, Fig. 4D).

4. Discussion

We designed this study to evaluate the effects of D,L-methadone on OC-cells in vitro, with the goal of shedding light on the drugs' potential beneficial but also detrimental effects on tumor growth.

In order to evaluate the plausibility of methadone's action on OC-cells, we analyzed first the expression of OPRM1, the main receptor and action site of methadone, in OC cell-lines and OC-tissues. Only one

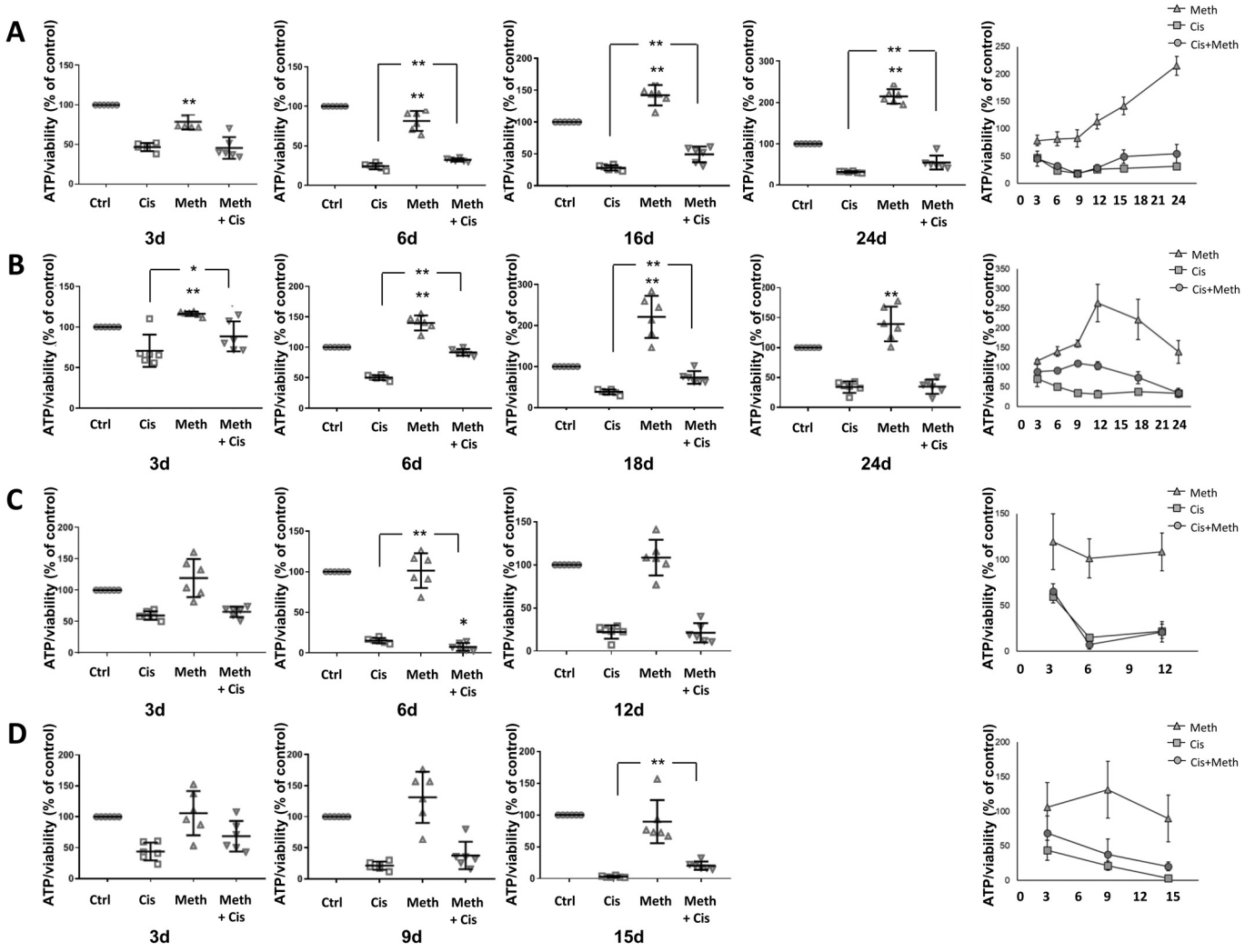

Fig. 3. Cellular viability of tumor-spheroids from HGSC-patients. Tumor-spheroids isolated from ascites from (A) a 78 years old OC-patient (B) a 61 years old OC-patient and tumor-spheroids derived from tumor specimens from (C) a 61 years old OC-patient and (D) a 54 years old OC-patient were treated with 10 μM cisplatin, 10 μg/ml methadone or a combination for the indicated days and cultivated in a bioreactor. Results from six independent experiments each are shown (± standard deviations). Statistical significance is indicated by asterisks representing p-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

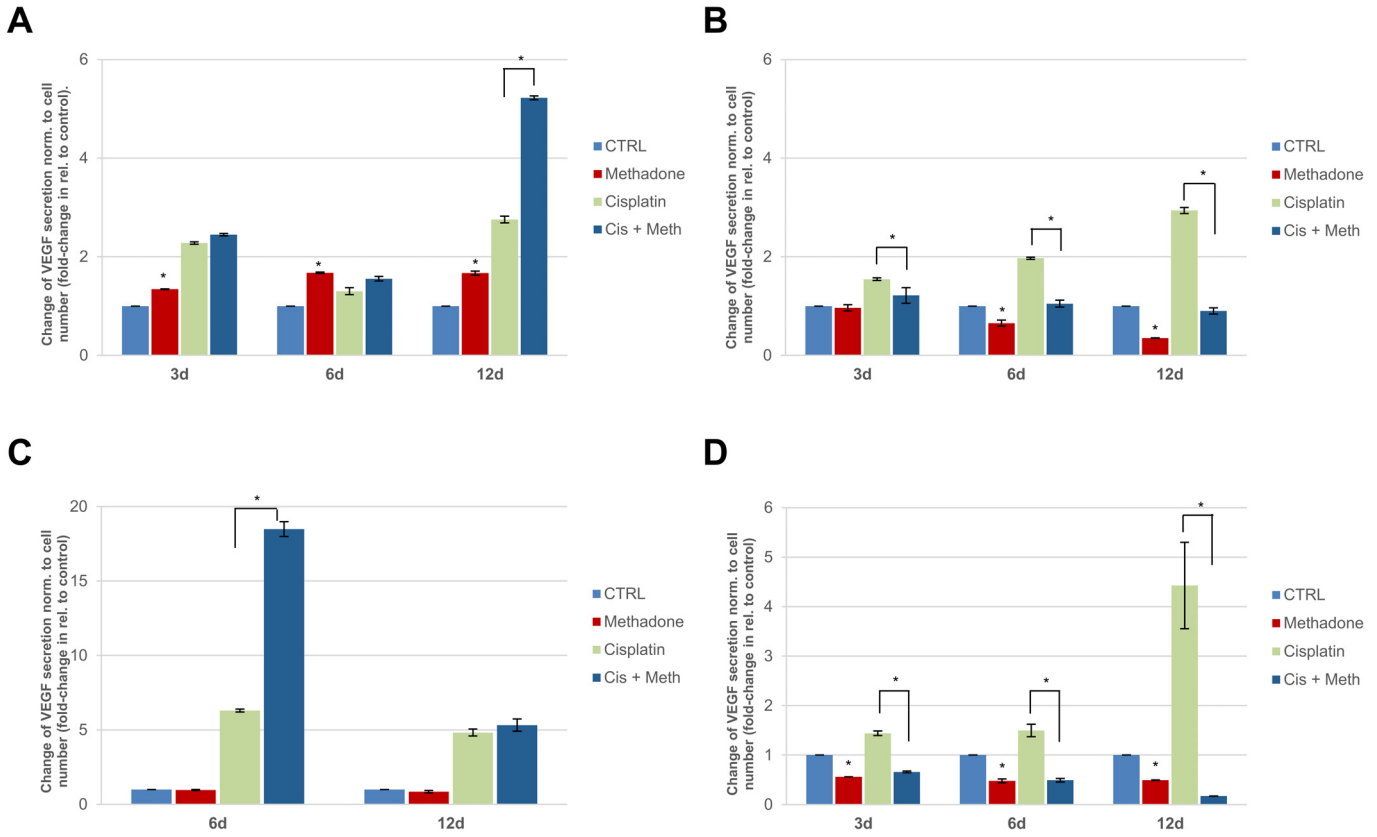

Fig. 4. VEGF-secretion of tumor-spheroids derived from ascites from HGSC-patients. Tumor-spheroids isolated from ascites from (A) a 78 years old OC-patient (B) a 61 years old OC-patient and tumor-spheroids derived from tumor specimens from (C) a 61 years old OC-patient and (D) a 54 years old OC-patient were treated with 10 μM cisplatin, 10 μg/ml methadone or a combination for the indicated days and cultivated in a bioreactor. Supernatants were collected and the VEGF concentration was measured. Results are presented as changes in relation to the untreated control cells and normalized to the cell number. Statistical significance is indicated by asterisks representing p-values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

OC cell-line out of eight showed OPRM1-expression at the mRNA and protein level. In 69% of the analyzed OC-tissues *OPRM1*-mRNA was detected though at a very low level, although the protein expression was not detectable. Our observation of the low abundance of OPRM1 in OC and non-neoplastic fallopian tubes is in accordance with the Human Protein Atlas, in which a high expression of *OPRM1*-mRNA has been described only in brain and testis [21]. The low levels of OPRM1 mRNA in the cell lines and tumor tissue suggest that this molecule is expressed by cells in the tumor microenvironment rather than by the tumor cells themselves. A single-cell transcriptomics dataset in the ovary published by the Human Protein Atlas, based on the data of Man et al., showed no expression in endothelial cells and only low RNA expression in smooth muscle cells and macrophages, although even in these cells the normalized transcript expression levels were below the cut-off value of 1 nTPM (consensus normalized expression) as the detection limit [22].

Nonetheless, the treatment with D,L-methadone reduced the cell-viability in OPRM1 negative and positive cell-lines. Therefore, we speculate that alternative pathways for opiate action beyond those known via the OPRM1 may exist and play an important role. Several authors have described possible mechanisms, through which opioids influence tumor growth, specifically via the activation of MAPK/ERK-pathway, the urokinase plasminogen activator (uPA) secretion and induction of epithelial mesenchymal transition (EMT) [23–29]. The exposure of the OC cell-lines to a combination of D,L-methadone with cisplatin in the treatment of the OC cell-lines did not show any biologically relevant, additive anti-oncogenic effects in the treatment of the OC cell-lines in comparison to the cisplatin therapy alone. Only in the A2780Cis an 11% stronger reduction of the cell-viability was observed with the combination treatment. This can possibly be explained by a re-sensitization to platinum, although this effect was not very pronounced.

Interestingly, the treatment with D,L-methadone alone had no inhibitory effect on the viability of the tumor-spheroids derived from minced tumor specimens from two patients at all assessment points. However, in ascites derived tumor-spheroids from two other patients in the majority of the assessment time-points even an undesired and worrisome increase in cellular viability was observed. The addition of D,L-methadone did not improve the cisplatin effect in three patients during long-term treatment, or even significantly worsened the cisplatin-induced reduction in cell viability at the end of therapy. Only in one patient a cisplatin-enhancing effect was observed during treatment, which, however, disappeared at the end of therapy. These growth stimulatory effects of D,L-methadone observed in the majority of our experiments are in accordance with published data showing proliferation promoting and tumor growth supporting effects of opioids [30].

Angiogenesis is a prominent hallmark of cancer. It requires a complex network between tumor cells and the surrounding microenvironment, which is tightly orchestrated by angiogenic factors including VEGF [31]. Therefore our experiments with D,L-methadone in patient-derived tumor-spheroids, where we observed both an increase and a decrease of the VEGF-secretion, mimic the heterogeneity of OC and seem to be more representative of in vivo conditions than the analysis of tumor cell-lines, where no effects on VEGF-secretion were detected. Analogously, Gupta et al. demonstrated in a human breast tumor xenograft model that morphine promoted tumor neovascularization, leading to increased tumor progression [23]. Direct immunosuppressive effects of opioids have also been shown in a mouse model where morphine gradually decreased the percentages of CD4+ and CD8+ T-lymphocytes and suppressed the proliferative abilities of T-lymphocytes [32].

However, the results obtained in published in vitro and in vivo studies are conflicting since both pro-apoptotic and anti-angiogenic properties of morphine have been demonstrated [9]. We would argue that the effects of opioids depend foremost on the tumor entity, realizing that there is a great heterogeneity within individual tumors. This is most likely the explanation for the varying results, which we obtained in this OC-study. Further investigations in mouse models could be helpful for the analyses of heterogeneous tumors like OC.

In conclusion, our results do not demonstrate a clear benefit to the administration of D,L-methadone as an adjuvant chemosensitizing anticancer drug in OC-patients, as no supportive effects were observed in most experiments in 2D and 3D cell cultures. To the contrary, in some 3D experiments we found detrimental effects of D,L-methadone, either by enhancing OC-cell proliferation as a single agent, or by abrogating the therapeutic effect of cisplatin. Therefore, OC-patients should be counseled and informed regarding the possible adverse effects of D,L-methadone. Unless future studies show otherwise, based on our results, we see no role for D,L-methadone as an adjuvant agent in ovarian cancer treatment.

Authors' contributions

H·F., and A.G.Z. developed the study concept, and designed the study. H.F., J.H., M.J.A., C.K., B.S., S.S., I.T., V.W., E.R., J.R., K.H., O.C., W.P., Su.S. were involved in data acquisition and quality control of data and algorithms, H.F., J.H., M.J.A., C.K., B.S., S.S., I.T., V.W., E.R., Su.S., C.M., A.G.Z. analyzed and interpreted data. H.F., J.H., C.K. performed statistical analyses. H.F prepared the manuscript. H.F., J.H., M.J.A., C.K., B.S., S.S., I.T., V.W., E.R., J.R., K.H., O.C., W.P., Su.S., C.M., A.G.Z. edited the manuscript and reviewed the final version.

Ethics approval

The study was reviewed and approved by the Ethics Committee of the Medical University of Innsbruck (reference no. 1054/2019) and conducted in accordance with the Declaration of Helsinki.

Consent for publication

Non applicable. The manuscript does not contain any individual person's data.

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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Financial supporter neither had any role in the design of this study, its execution, analyses or interpretation of the data, nor in the decision to submit results for publication.

Declaration of Competing Interest

The authors have declared no conflicts of interest.

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