Stimulated upregulation of *HO-1* is associated with inadequate response of gastric and ovarian cancer cell lines to hyperthermia and cisplatin treatment

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Abstract. Heme oxygenase (HO)-1 is a heat shock protein induced by hyperthermia, responsible for cellular resistance to temperature. The aim of this *in vitro* study was to clarify the response of gastric and ovarian cancer cells to hyperthermic intraperitoneal chemotherapy, following the modulation of *HO-1* expression. AGS and OVCAR-3 cells were treated with different temperature regimens, either alone or in combination with an IC_{50} dose of cisplatin for 1 h. Prior to treatment, *HO-1* expression was silenced by short interfering RNA transfection. In OVCAR-3 cells, cisplatin increased *HO-1* mRNA expression by 3.73-fold under normothermia and 2.4-fold under hyperthermia; furthermore, these factors similarly increased *HO-1* protein expression levels. Exposure to cisplatin under hyperthermia reduced the viability of OVCAR-3 cells by 36% and *HO-1*-silencing enhanced this effect by 20%. *HO-1*-silencing under normothermia increased apoptotic rates in cisplatin-treated OVCAR-3 cells by 2.07-fold, and hyperthermia enhanced the effect by 3.09-fold. Semi-quantitative polymerase chain reaction (PCR) cell analysis indicated that exposure to cisplatin decreased the cell index under normothermia, and that hyperthermia boosted this effect in OVCAR-3. In AGS cells, only temperature increased cellular *HO-1* levels. Silencing *HO-1* in AGS cells at 37˚C reduced viability by 16% and increased apoptotic rates 2.63-fold. Hyperthermia did not affect AGS viability; however, apoptosis was increased 6.84-fold. PCR analysis indicated no additional effects of hyperthermia on the AGS cell index. *HO-1* is induced in cancer cells by different stressors in a variable manner. In tumors with highly inducible *HO-1*, prior silencing of this gene could improve the cellular response to hyperthermia and cisplatin.

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Introduction

The treatment of cancer in the peritoneal cavity is a major problem in gastrointestinal and gynecological oncology worldwide (1,2). Globally, patients with gastric cancer with peritoneal carcinomatosis have a median survival time of 3-6 months, when the disease is left untreated (3). In the majority of newly diagnosed ovarian cancer cases, peritoneal metastases are already present (4).

Hyperthermic intraperitoneal chemotherapy (HIPEC) is a promising treatment option for intraperitoneally disseminated cancer (5-7). It has been reported to be more efficient compared with intravenous chemotherapy, however, as a treatment of peritoneal metastases it is limited by the plasma-peritoneal barrier (8,9). It has been previously suggested that hyperthermia can enhance the intraperitoneal application of cytotoxic agents (10,11). Residual microtumors, following cytoreductive surgery, are treated by intraperitoneal chemotherapy in combination with hyperthermia up to 42-43˚C (12). Overall, hyperthermia as part of HIPEC is thought to boost pharmacokinetics and increase DNA damage induced by the cytostatic agent (13). Cisplatin is an alkylating drug used to treat residual gastric and ovarian cancer in the peritoneal cavity (14). Previous *in vitro* studies produced controversial data about the additivity of hyperthermia to cisplatin (15,16).

The response and resistance of cancer cells to chemotherapy and hyperthermia depend on the induction and expression of a number of cytoprotective proteins, including Hsp70 and Hsp27 (17-19). Therefore, the modulation of cytoprotective proteins may serve a crucial role in cancer treatment. One potential target is heme oxygenase (HO)-1, particularly in HIPEC, since high temperatures are a component of HIPEC, and it has been reported that, under hyperthermia, cells enhance *HO-1* expression for self-protective purposes (19). *HO-1* is normally expressed at low levels in the majority of tissues, including the gastrointestinal tract, female reproductive organs, brain and bone marrow (20); however, it is highly inducible by a variety of stimuli, including cytokines, lipopolysaccharides (21) and

serine/threonine kinases (22). Cellular levels of *HO-1* are known to be temperature-dependent (23,24). *HO-1* is overexpressed under hyperthermic conditions, exerting a protective function (25,26).

An *in vitro* study was conducted to clarify the underlying mechanism of how cisplatin and hyperthermia induce *HO-1* expression in ovarian and gastric cancer cells. In addition, the present study investigated the response of these cancer cells to cisplatin and hyperthermia following the modulation of *HO-1* protein expression.

Materials and methods

Cell lines and conditions. Human gastric adenocarcinoma, AGS, and ovarian adenocarcinoma, OVCAR-3, cell lines were purchased from the American Type Tissue Culture Collection (Manassas, VA, USA). OVCAR-3 cells were cultivated in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 20% fetal bovine serum (FBS; Gibco; Thermo Fisher Scientific, Inc.), 1% penicillin/streptomycin and 0.01 mg/ml bovine insulin (Gibco; Thermo Fisher Scientific, Inc.). AGS cells were harvested in Ham's F-12K medium with 10% FBS and 1% penicillin/streptomycin at 37˚C and 5% CO_2.

Experimental design. Cells were harvested for 24 h in the conditions described previously. The cells were subjected to conditions of normothermia (37˚C) or 43˚C and/or an IC_{50} dose of cisplatin. The IC_{50} dose was determined for each cell line individually in an experimental manner. The IC_{50} doses of cisplatin for AGS and OVCAR-3 cells were determined (at 37˚C) to be 111 and 152 μM, respectively. Hyperthermia and/or cisplatin exposure lasted for 1 h; this step began once the media reached the desired temperature (37 or 43˚C), as measured by a digital thermometer in a humid incubator with a set temperature of 43˚C. Following treatment, the medium was changed and cells were harvested after 48 h of incubation in a humidified atmosphere at 37˚C with 5% CO_2. AGS and OCAR-3 cell viability, apoptosis, and cell index were all subsequently measured. Additionally, these cell lines were used in real time cell analysis, western blotting and semi-quantitative polymerase chain reaction (qPCR) assays.

Silencing of HO-1. *HO-1* small interfering RNA (siRNA; 30 nM HMOX1; sense 5'-UGAACACUCUGGAGAUGAC-3', and antisense 5'-GUCAUCUCCAGAGUGUCCA-3') was obtained from Ambion; Thermo Fisher Scientific, Inc., and negative control (30 nM AllStars Negative Control siRNA; sense 5'-UUCUCCGAACGUGUCACGU-3' and antisense 5'-ACGUGACACGUUCGGAGAA-3') was obtained from Qiagen GmbH (Hilden, Germany). Lipofectamine® RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc.) and Opti-MEM™ media (Gibco; Thermo Fisher Scientific, Inc.) were used according to the manufacturer's protocols. The efficiency of transfection was verified using BLOCK-iT Alexa Fluor (Invitrogen; Thermo Fisher Scientific, Inc.). The efficiency of knockdown was verified by western blot analysis. *HO-1*-silencing was performed 72 h prior to implementation of the experimental temperature and treatment with cisplatin.

MTT assay. Cell viability was determined using an MTT assay (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were incubated for 4 h at 37˚C following the addition of 5 mg/ml MTT reagent. The supernatant was subsequently removed and dimethyl sulfide was added (Carl Roth GmbH Co KG, Karlsruhe, Germany). Absorbance was measured at a wavelength of 570 nm and the reference was measured at 650 nm using a Sunrise spectrophotometer (Tecan Austria GmbH, Grödig, Austria).

qPCR. Cellular RNA was extracted using a PureLink® RNA Mini kit (Ambion; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols. Purified RNA was measured and verified for purity using ultraviolet (UV) spectrophotometry (NanoDrop; Thermo Fisher Scientific, Inc.). Using the Super Script Vilo Master Mix (Invitrogen; Thermo Fisher Scientific, Inc.) with 2 μg RNA, cDNA was generated, according to the manufacturer's protocols. RNA amplification was performed in a 20 μl reaction volume, which contained 1X PCR Master Mix, primers, and 2 μl cDNA template. Thermocycling conditions were as follows: initial step at 95˚C for 10 min (1 cycle), denaturation at 95˚C for 15 sec and annealing/extending at 60˚C for 1 min (40 cycles), followed by a final extension step at 72˚C for 2 min. *HO-1* primers were obtained from Invitrogen (Thermo Fisher Scientific, Inc.): forward, 5'-TGCTCAACATCCAGCTCTTTGAGGA-3'; and reverse, 5'-CAGGCAGAGAATGCTGAGTTC-3'. The products were loaded on 1.5% agarose gels. Ethidium bromide staining and UV light (Gel Doc™ XR+ Gel Documentation System; Bio-Rad Laboratories, Inc., Hercules, CA, USA) were used for visualization. Analysis was performed using ImageLab software (version 6.0.0; Bio-Rad Laboratories, Inc.).

Flow cytometry. Apoptosis was evaluated by flow cytometry using Annexin V-PE and 7-aminoactinomycin D. A Guava Nexin Annexin V Assay kit (Merck KGaA, Darmstadt, Germany) was used according to the manufacturer's protocols. Analysis was performed with the Guava Personal Cell Analysis Flow Cytometer (Guava; EMD Millipore, Billerica, MA, USA) and CytoSoft software (version 2.1.4; Guava; EMD Millipore).

Western blot analysis. Lysates were prepared using radioimmunoprecipitation lysis buffer (Abcam, Cambridge, UK) containing protease inhibitors (Roche Diagnostics, Basel, Switzerland). A bicinchoninic acid protein assay kit (Thermo Fisher Scientific, Inc.) was used to determine the protein concentration, according to the manufacturer's protocols. Following heating at 97˚C for 5 min, protein samples (50 μg) were subjected to 4-12% SDS-PAGE and transferred to polyvinylidene fluoride membranes at 30 V for 50 min. Membranes were blocked with a blocking buffer (20% diluent A, 30% diluent B; WesternBreeze Blocker/Diluent; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 1 h and incubated with the primary antibodies rabbit anti-*HO-1* (dilution, 1:2,000; cat. no., EP1391Y; Abcam) and mouse anti-GAPDH (dilution, 1:1,000; cat. no., AM4300; Ambion; Thermo Fisher Scientific, Inc.) at 4˚C overnight. The following day, the blots were incubated with ready to use secondary antibodies against rabbit (cat. no. WP20007; Invitrogen, Thermo Fisher Scientific, Inc.)

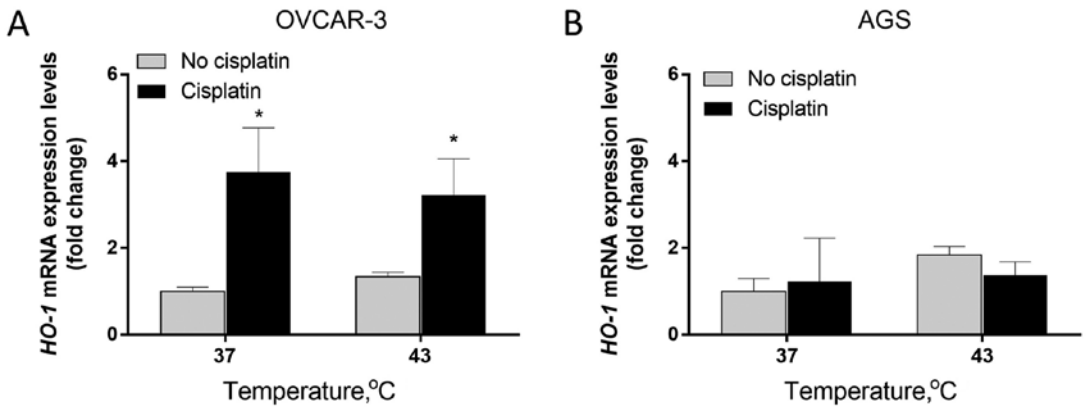

Figure 1. *HO-1* expression is differentially induced by hyperthermia and cisplatin treatment in ovarian adenocarcinoma, OVCAR-3, and gastric adenocarcinoma, AGS, cell lines. (A) *HO-1* expression was activated by cisplatin in OVCAR-3 cells, whereas hyperthermia was not indicated to have an additional effect. (B) In AGS cells, only hyperthermia indicated to induce *HO-1* expression. *P<0.05, compared with the control group (not treated with cisplatin, normothermia). *HO-1*, heme oxygenase-1.

or mouse immunoglobulin G (cat. no. WP20006; Invitrogen; Thermo Fisher Scientific, Inc.) for 1 h at room temperature. Chemiluminescence substrate (CDP-Star; Invitrogen; Thermo Fisher Scientific, Inc.) was added and the ChemiDoc imaging system (Bio-Rad Laboratories, Inc.) was used for visualization. ImageJ software (version 1.48; National Institutes of Health, Bethesda, MD, USA) was used for quantification of western blots (27).

Real time cell analysis. The xCELLigence® RTCA DP Real-Time Analyzer (ACEA Biosciences Inc., San Diego, CA, USA) was used to investigate the PCR cellular response to treatment. To present the real time cell analysis data obtained by xCELLigence system, the cell index was used. The determination of the cell index parameter is an automatic system feature and is based on the rapid measurements of impedance between gold electrodes in the analyzer wells (28). Cell-free medium (RPMI-1640; Gibco; Thermo Fisher Scientific, Inc.) and medium with cells have different cell impedance and proliferation indices, which is reflected in changes in impedance value and CI; this is calculated as follows: (impedancetime point n-impedancebackground)/nominal impedance value. Delta cell index was measured immediately when cells reached the electronic microplate. Cells were evaluated for 48 h following the experiment and/or 72 h following *HO-1*-silencing.

Statistical analysis. SPSS v.21.0 software (IBM Corp., Armonk, NY, USA) and GraphPad (version 6.01; GraphPad Software Inc., La Jolla, CA, USA) were used for statistical evaluation. The Mann-Whitney test and one-way analysis of variance with a Bonferroni post hoc test were performed to assess clinical significance. Data are presented as the mean ± standard deviation of three independent experiments, performed in triplicate. P<0.05 was considered to indicate a statistically significant difference.

Results

Hyperthermia and cisplatin differentially induce HO-1 mRNA and protein expression in ovarian and gastric cancer cells. Enhanced expression levels of *HO-1* mRNA were only observed in OVCAR-3 cells. The exposure of OVCAR-3 cells to cisplatin resulted in a significant increase of *HO-1* mRNA expression. Cisplatin induced a 3.75- and 2.4-fold increase of *HO-1* expression in conditions of normothermia (37˚C) and hyperthermia (43˚C), respectively (P<0.05). While hyperthermia at 43˚C boosted *HO-1* expression by 1.34-fold, the addition of cisplatin increased the effect on *HO-1* expression by 3.22-fold (P<0.05; Fig. 1A). In AGS cells, *HO-1* expression was not significantly affected by temperature or cisplatin (Fig. 1B).

Furthermore, cisplatin significantly increased *HO-1* protein expression in OVCAR-3 cells (P<0.05). *HO-1* expression was increased 9.5-fold, following cisplatin treatment under normothermia. At 43˚C, this effect was slightly higher, with a 9.77-fold increase (P<0.05). The exposure of OVCAR-3 cells to hyperthermia alone had no effect on *HO-1* protein expression levels. However, the combination of cisplatin and hyperthermia increased *HO-1* protein expression by 11-fold compared with the control (untreated cells in normothermia; P<0.01; Fig. 2A).

In AGS cells, cisplatin had no notable effect on *HO-1* expression. At 37˚C, cisplatin increased *HO-1* protein expression in AGS cells by 1.2-fold; however, this increase was not statistically significant, whereas the exposure of cells to 43˚C in the absence of cisplatin increased *HO-1* protein expression levels by 2.75-fold (P<0.05). *HO-1* expression dropped slightly when cisplatin was added at 43˚C. Therefore, concomitant treatment of AGS cells with cisplatin and hyperthermia at 43˚C resulted in a 2.14-fold increase in *HO-1* protein compared with the control (Fig. 2B). Furthermore, *HO-1* knockdown was assessed by western blotting (Fig. 2C and D).

HO-1-silencing does not influence AGS cell viability. The results of the MTT assay revealed that *HO-1*-silencing in OVCAR-3 cells does not affect viability in response to cisplatin at 37˚C. The exposure of OVCAR-3 cells to cisplatin and hyperthermia (43˚C) resulted in a 36% drop in cell viability (P<0.05). *HO-1*-silencing enhanced this effect by an additional 20% (P<0.05; Fig. 3A).

HO-1-silencing in AGS cells enhanced the cisplatin effect and reduced cell viability by 16% at 37˚C (P<0.05).

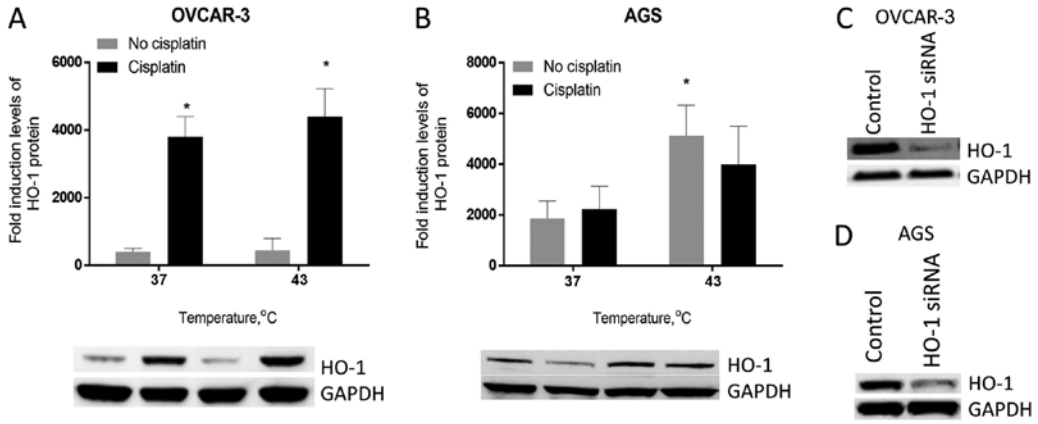

Figure 2. *HO-1* protein expression is activated by cisplatin or hyperthermia. (A) *HO-1* expression increased following cisplatin treatment of OVCAR-3 cells. Hyperthermia-dependent activation of *HO-1* expression did not indicate any significant differences between 37 and 43˚C. (B) In AGS cells, hyperthermia induced expression of HO-1, while treatment with cisplatin had no significant effect. HO-1-knockdown decreased expression levels of *HO-1* protein in (C) OVCAR-3 and (D) AGS cells compared with corresponding cells treated under normothermia and without cisplatin. *P<0.05, compared with the control group (not treated with cisplatin, normothermia). Western blotting data is shown by selecting the most representative experiment. *HO-1*, heme oxygenase-1; siRNA, small interfering RNA.

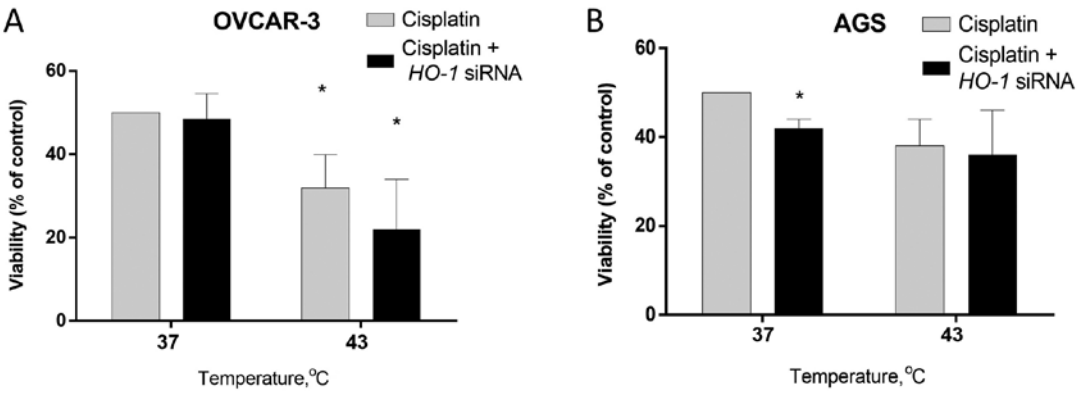

Figure 3. Modulating *HO-1* expression differentially influences the viability of OVCAR-3 cells, however not AGS cells. (A) *HO-1*-silencing had no significant effect on the viability of OVCAR-3 cells treated with cisplatin in conditions of normothermia. *HO-1*-silencing enhanced the reduction of cell viability, following treatment with cisplatin and hyperthermia. (B) *HO-1*-silencing slightly decreased AGS cell viability, following treatment with cisplatin under normothermia, however there was no significant effect with hyperthermia. *P<0.05, compared with the cells treated with cisplatin under normothermia. Data are equalized to untreated cells-100%. *HO-1*, heme oxygenase-1; siRNA, small interfering RNA.

Hyperthermia potentiated cisplatin cytotoxicity in AGS cells: viability dropped by 24% compared with 37˚C. However, *HO-1*-silencing had no significant additional effect, whereas viability rates were similar in *HO-1*-silenced or unsilenced AGS cells following cisplatin treatment at 43˚C (Fig. 3B).

HO-1-silencing prior to concomitant hyperthermia and cisplatin treatment increases ovarian and gastric cancer cell apoptosis. The exposure of OVCAR-3 and AGS cells to hyperthermia resulted in a better cell response to cisplatin with respect to apoptosis. Prior *HO-1*-silencing under normothermia increased cisplatin-induced apoptosis in OVCAR-3 and AGS cells by 2.07- and 2.63-fold, respectively. In addition, silencing of *HO-1* under hyperthermia enhanced the apoptosis of OVCAR-3 and AGS cells by 3.09- and 6.84-fold, respectively (P<0.05; Fig. 4).

Hyperthermia enhances the effect of cisplatin on OVCAR-3, but not on AGS cells, following modulation of HO-1 expression. PCR analysis for 48 h following treatment indicated that exposure to cisplatin resulted in a gradual decrease in the cell index of AGS and OVCAR-3 (*HO-1*-silenced) cells at 37˚C. Hyperthermia at 43˚C boosted this effect by inducing a gradual decrease of the OVCAR-3 (*HO-1*-silenced) cell index. However, the cell index of AGS (*HO-1*-silenced) cells following cisplatin treatment at 37 or 43˚C remained similar (Fig. 5).

Discussion

In the present study, the *HO-1* protein was variably expressed at the basal level and variably induced following exposure to cisplatin and hyperthermia in OVCAR-3 and AGS cells. Cisplatin increased the expression levels of *HO-1* in OVCAR-3 cells, while hyperthermia at 43˚C had no effect. In AGS cells, *HO-1* expression was slightly increased under hyperthermia, with no significant induction following exposure to cisplatin, indicating that the modulation of *HO-1* may serve a role in the response of cancer cells to cisplatin and hyperthermia and affect cancer treatment outcomes.

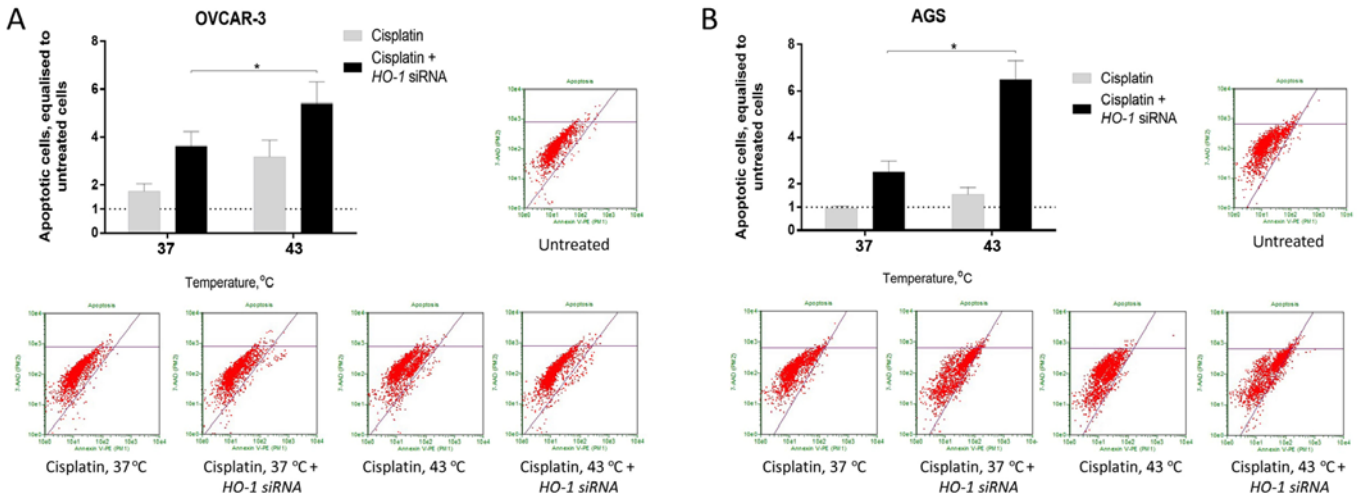

Figure 4. Prior HO-1-silencing and subsequent hyperthermia/cisplatin treatment enhances apoptosis in OVCAR-3 and AGS cells. (A) *HO-1*-silencing enhanced apoptosis in OVCAR-3 cells treated with cisplatin at 37˚C. This effect was even more evident under conditions of hyperthermia. (B) *HO-1*-silencing significantly enhanced apoptosis in cisplatin-treated AGS cells under hyperthermia compared with normothermia. Dot plots represent the raw data of the corresponding bar. Dashed lines indicate apoptosis in control (untreated) cells. *P<0.05. The data were obtained from five independent experiments. *HO-1*, heme oxygenase-1; siRNA, small interfering RNA.

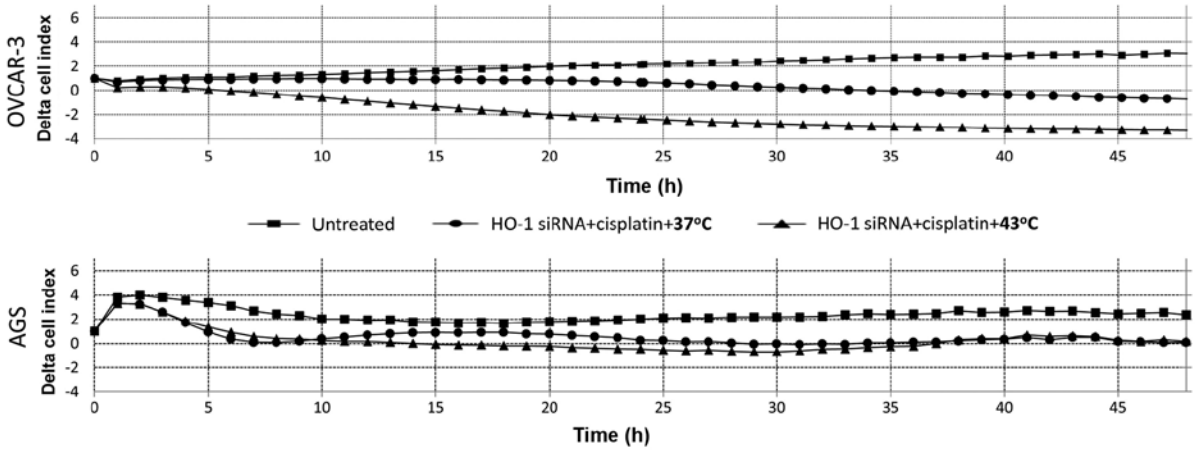

Figure 5. Hyperthermia enhances the effect of cisplatin only on OVCAR-3 cells. Exposure to cisplatin gradually decreased the cell index of OVCAR-3 and AGS (*HO-1* silenced) cells under normothermia. Hyperthermia enhanced this effect in OVCAR-3 cells, while the AGS cell index did not change. The data are shown by selecting the most representative experiment. *HO-1*, heme oxygenase-1; siRNA, small interfering RNA.

HIPEC is widely used in clinical settings, and promising results have been reported in the treatment of peritoneal dissemination of gastric and ovarian cancer (29,30). To the best of our knowledge, to date, there has been a lack of evidence regarding the synergy of chemotherapy and hyperthermia. In our previous studies, it was observed that gastrointestinal and ovarian cancer cells responded unpredictably following exposure to cisplatin and hyperthermia (16,31). One of the limits of this response may be the induction of cytoprotective enzymes associated with chemotherapy and/or hyperthermia, in particular *HO-1*. *HO-1* is known to be highly expressed in human gastric and ovarian cancer tissue (32). Anticancer treatment options, including chemotherapy and radiotherapy may increase *HO-1* expression (33). *HO-1* serves an important role in a number of pathophysiological conditions, including temperature rise and inflammation, and has been reported to be associated with cancer (34,35). *HO-1* expression is associated with cancer growth and progression by promoting angiogenesis in the tumor itself and metastases and pro-proliferation in different types of tumors, including renal cell carcinoma, prostate and pancreatic cancer, melanoma, and hepatoma (36-40). Numerous studies have highlighted that cancer cells with high expression levels of *HO-1* are less sensitive to cisplatin treatment compared with cancer cells with low *HO-1* expression levels (41,42).

The mechanism underlying this cytoprotective effect relies on the ability of *HO-1* to catabolize free heme and prevent it from sensitizing cells to undergo programmed cell death (43). *HO-1* under normal conditions has various cellular functions, including catalyzing the heme molecule to form bile pigments (44). When stimulus (heat) is present, cellular *HO-1* synthesis is enhanced (45). Therefore, the present study suggests that *HO-1* is crucially important when dealing with intraperitoneally-spread cancer, particularly treating it with HIPEC. Following the administration of heated chemotherapy drugs into the abdominal cavity, tumor cells should start to defend themselves, by activating heat shock proteins, particu-

larly *HO-1*. The aim of the present study is to achieve better treatment results by downregulating *HO-1* expression.

To the best of our knowledge, there are no published data on the efficacy of HIPEC treatment while modulating the expression of *HO-1*. The results of our study demonstrate the impact of *HO-1* expression modulation in the combination treatment of hyperthermia at 43˚C and cisplatin in OVCAR-3 cells.

Zhao *et al* (46) reported that the basal level of *HO-1* expression is higher in ovarian cancer cells compared with normal ovarian tissues. A high level of *HO-1* expression has also been associated with aggressive tumors and poor clinical outcomes (46). The ability of cisplatin to increase the expression of *HO-1* was also observed in different cancer types, including pancreatic and hepatic cancer (43,47,48). Was *et al* (37) reported the different abilities of tumor tissues to produce heat shock proteins. Nonetheless, a high level of *HO-1* is known to be associated with the reduced tumor growth observed in some types of cancers, including breast and prostate cancer and non-small-cell lung carcinoma (37).

The results of the present study indicate that the viability of *HO-1*-silenced OVCAR-3 cells decreased significantly following cisplatin treatment at 43˚C. However, in AGS cells, the inhibition of *HO-1* did not improve the response to cisplatin treatment. These results are associated with the expression of *HO-1*. It is possible that the inhibition of *HO-1* only increases the effect of cisplatin in cancer cells, where *HO-1* is highly expressed. This is in accordance with the data reported by Lv *et al* (41), where cisplatin significantly induced the expression of *HO-1*. The study modulated *HO-1* expression using hemin (an inducer of *HO-1*) and ZnPPIX (an inhibitor of *HO-1*), and reported that hemin strongly inhibited cisplatin-induced cell death, while ZnPPIX significantly increased cell death (41,49). These effects following *HO-1* modulation can be explained by the cytoprotective ability of this protein. *HO-1* activates a cellular defense mechanism against oxidative stress through its catalytic products, including ferrous iron, carbon monoxide, and biliverdin (37). In addition, growing evidence has suggested that *HO-1* protects cells from chemotherapeutic agent-induced apoptosis, and the targeted knockdown of *HO-1* gene expression or suppression of *HO-1* activity *in vitro* significantly enhances the chemosensitivity of cancer cells (50). Furthermore, it has been reported that the inhibition of *HO-1* can increase cellular response to anticancer treatment (26).

Cisplatin can effectively induce and promote apoptosis in a wide range of solid tumors, including head and neck cancer, esophageal carcinoma, non-small cell lung carcinoma, and testicular, cervical, and ovarian cancer (51). Inhibition of *HO-1* may strengthen the pro-apoptotic effects of cisplatin (41). In the present study, the inhibition of *HO-1* increased the number of apoptotic cells in OVCAR-3 and AGS cell lines, however these results did not indicate any significant differences associated with *HO-1* expression and cell viability. This could be explained by the fact that the present study measured the number of cells in both early and late apoptosis, and early apoptosis can be reversible (52). Geske *et al* (52) reported that the early stages of apoptosis are reversible if the apoptotic stimulus is removed, which is the reason that PCR analysis was performed in the present study.

In this experimental model, hyperthermia alone did not induce the upregulation of *HO-1* expression in the tested cancer cell lines. Nevertheless, *HO-1*-silencing resulted in the optimal response to cisplatin treatment in terms of cell viability in OVCAR-3 cells, and apoptosis in both OVCAR-3 and AGS cells, under conditions of hyperthermia. Therefore, a novel finding regarding the role of *HO-1* in HIPEC is presented in this study. In conclusion, the cytoprotective protein *HO-1* is induced in cancer cells by different stressors in a variable manner. In tumors with highly inducible *HO-1*, the present study indicated that prior silencing of this gene may significantly improve the cellular response to hyperthermia and cisplatin.

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Availability of data and materials

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

Authors' contributions

VC wrote the manuscript and analyzed the data. AS performed the western blot analysis and analyzed the data. GS and AJ performed semi-quantitative PCR and siRNA transfection. SP performed the PCR cell analysis and analyzed the data. ZD and AG revised the data and manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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