

Effects of cisplatin in combination with hyperthermia on biological characteristics of retroperitoneal liposarcoma

Cheng-Li Miao¹, Shahbaz Hanif², Lin Zhang³, Xiao-Bing Chen¹, Mei Huang¹, Shi-Bo Liu¹, Cheng-Hua Luo¹

¹Department of Retroperitoneal Tumor Surgery, Peking University International Hospital, Beijing 102206, China;

²Beijing Spanal Medical Scientific Co. Ltd., Beijing 102206, China;

³Molecular Diagnosis Laboratory, Department of Regenerative Medicine, Affiliated Hospital of Qingdao University, Qingdao, Shandong 266000, China.

Pathologically, retroperitoneal liposarcoma (RPLS) is a group of malignant tumors, which originates from adipose tissue in retroperitoneal space, accounting for almost 70% of all retroperitoneal tumors. RPLS has no optimal treatment. Surgical resection (R₀/R₁) is the most effective therapy; however, recurrence rate is very high. Grossly incomplete surgical resection (R₂) is an important risk factor for early recurrence of RPLS.^[1]

Cisplatin, the most frequently used platinum-based chemotherapeutic agent, plays a significant role in the treatment of various malignancies. Generally, hyperthermia (HT) can heat the whole body or a specific area of the body above the normal temperature, so that curative effects on solid tumors may be achieved. Malignant cells are extremely sensitive to heat. Mechanically, HT inhibits DNA damage repair system in cancer cells. How to set-up HT temperature relies on specific purposes of treatment. Suitable HT can directly eradicate tumor cells without damaging nearby normal tissue. To kill tumor cells thoroughly, the temperature of HT can be kept as high as 80°C. Whereas, to kill tumor cells only, without harming the adjacent organs and tissues, the temperature of HT can be adjusted to 41 to 45 °C.

Simultaneously, HT has the potential to augment efficacy of chemotherapy or radiotherapy. HT can attenuate side-effects of combinatory therapies, reduce drug resistance, promote apoptosis of tumor cells, and enhance cytotoxicity of chemotherapy, including cisplatin.^[2] To improve therapeutic efficacy, combinatory use of HT with cisplatin is typically applied to treat various cancers, mainly those with poor prognosis (eg, soft tissue sarcoma, colon, bladder, and liver cancer). However, potential roles of cisplatin and HT, either alone or in combination, in RPLS have never been addressed. Their influence on morphology and proliferation of RPLS cells also remained uninvestigated. Therefore, to fill the gaps in knowledge, we

examined morphology and proliferation of RPLS SW872 cell line in response to cisplatin and HT, either alone or in combination. In addition, molecular mechanism was explored.

The human RPLS SW872 cell line was purchased from American Type Culture Collection (ATCC, Maryland, USA). Cells were removed from liquid nitrogen and quickly dispersed in a 37°C water bath. Then, the cells were transferred to a complete medium containing 10% fetal bovine serum (FBS) and cultured in a CO₂ cell incubator. On the following day, the cells were attached and presented in spindle shaped. SW872 cells were maintained in logarithmic growth stage. Cell density was adjusted to 1 × 10⁵/mL. Cells (100 μL/well) were seeded to measure plating efficiency. SW872 cells were grown in a 96-well tissue culture plate and incubated at 37°C in a 5% CO₂-saturated humidified incubator for 24 h. Afterward, culture medium was discarded; cells were mixed with Dulbecco modified Eagle medium containing 10% FBS and cisplatin. Cisplatin concentration range for primary screening was 1.25, 2.5, 5, 10, 20, 40, and 80 μg/mL. In another group, Ferostatin-1 (Fer-1), an iron-dependent cell death inhibitor was added to cisplatin at a concentration of 1 μmol/L. Cell counting kit-8 (CCK-8) was used to detect cytotoxicity of cisplatin to SW872 cells after incubation for 24 h. A 10 μL CCK-8 was added to each well, and absorbance at 450 nm was detected by enzyme label after incubation at 37°C for 3 h. Cell growth inhibition rate was calculated, and the concentration of cisplatin with an inhibition rate of 30% to 50% was selected as sub-cytotoxic dose for further experiments.

Apoptosis was detected by flow cytometry using Annexin V-FITC kit (Abcam, ab14085, UK). When reaching 70% to 80% of confluence, SW872 cells were treated with HT. The cells were digested with 0.25% trypsin in phosphate buffer saline and adjusted to a concentration of 10⁶/mL. One

Access this article online

Quick Response Code:



Website:

www.cmj.org

DOI:

10.1097/CM9.0000000000001326

Cheng-Li Miao and Shahbaz Hanif contributed equally to this work.

Correspondence to: Cheng-Hua Luo, Department of Retroperitoneal Tumor Surgery, Peking University International Hospital, Beijing 102206, China
E-Mail: luochenghua@pku.edu.cn

Copyright © 2020 The Chinese Medical Association, produced by Wolters Kluwer, Inc. under the CC-BY-NC-ND license. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Chinese Medical Journal 2021;134(9)

Received: 12-05-2020 Edited by: Li-Shao Guo

hundred microliters of cell suspension was centrifuged at 1000 r/min for 5 min at 4°C. The supernatant was discarded. Then, cells were treated with 200 μ L binding buffer, 5 μ L propidium iodide, and 5 μ L Annexin-V. Cells were kept at room temperature for 20 min. Afterward, 400 μ L binding buffer was added to detect apoptosis with flow cytometry.

SW872 cell lysate was used to extract total protein. Protein content was determined by bicinchoninic acid quantitative kit (Thermo Scientific, Phadia AB, Uppsala, Sweden). Preliminary experiment was carried out to determine an optimal sample size. Protein samples were denatured by boiling at high temperature. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis electrophoresis, samples were incubated for 12 h with primary antibodies at 4°C, and incubated with secondary antibodies in a shaking table for 2 h. After treated with electrochemiluminescence prime, protein density was analyzed by gel imager.

SW872 cells at logarithmic growth stage were incubated in 96-well plates at 37°C for 4 h (37-4 group), at 42°C for 0.5 h and then at 37°C for 3 h (42-0.5-3 group), at 42°C for 1 h and then at 37°C for 3 h (42-1-3 group), at 37°C for 7 h (37-7 group), at 42°C for 0.5 h and then at 37°C for 6 h (42-0.5-6 group), or at 42°C for 1 h and then at 37°C for 6 h (42-1-6 group). Total RNA was extracted by Trizol reagent, and transcribed to complementary DNA. Target gene was amplified by quantitative real time polymerase chain reaction with the following primers: tumor necrosis factor (TNF)- α upstream: 5'-CAACCTCAGACGCCA-CATC-3', downstream: 5'-CCTTTCCAGGGAGGGGG-GAGGGG-3'; GPX4 upstream: 5'-CGCTGTGGAAGTG GATAG-3', downstream: 5'-GAGCTAGAAATAGGTGG GVAGGT-5'. Reaction parameters were set up as follows: pre-denaturation at 95°C for 2 min, denaturation at 95°C for 15 s, annealing at 60°C for 15 s, and extension at 72°C for 30 s, 40 cycles. The data were analyzed by $2^{-\Delta\Delta C_t}$ method using β -actin as internal control.

Dynamic morphological changes were observed and photographed under an inverted microscope ($\times 400$) according to the experimental design. For control group, RPLS cells received no treatment. For HT group, RPLS cells were heated at 41°C for 0.5 h, and cultured routinely. For chemotherapy group, RPLS cells were treated with cisplatin and cultured routinely for 24 h. For thermochemotherapy group, RPLS cells were treated with cisplatin, followed by heated at 41°C for 0.5 h and then cultured routinely for 24 h.

SW872 cells were cultured in 96-well plates containing 10% FBS medium with 5×10^3 /well for 12 h, and then treated with different conditions. For control group, cells received no treatment. For HT group, RPLS cells were incubated at 41 and 43°C for 0.5 and 1 h, respectively. For chemotherapy group, RPLS cells were treated with cisplatin (5 μ g/mL). For thermochemotherapy group, RPLS cells were treated with cisplatin, followed by heated at 41 or 43°C for 0.5 or 1 h, respectively. CCK-8 was used to determine cell viability after 12 h of culture. Growth inhibition rate was calculated and absorbance value *A* was recorded. Cell growth inhibition rate was equal to $(1 - A \text{ value of experimental group} / A \text{ value of cell control group}) \times 100\%$.

Results are expressed as mean \pm standard deviation (SD). All statistical analyses were performed using SPSS 24.0 software (IBM, USA). One-way analysis of variance and *T*-test were used for comparison between groups. Statistical significance was defined as $P < 0.05$.

Cytotoxicity of cisplatin at different concentrations was detected by CCK-8 assay. With an increase in cisplatin concentration, cytotoxicity gradually increased. At 5 μ g/mL, the inhibition rate of cisplatin on cell viability reached 50%. Fer-1 could not reverse DNA damage induced by cisplatin. HT promoted apoptosis. The apoptotic and cell death rates were 51.8% and 41.6%, respectively, after HT at 41°C for 0.5 h. Similarly, apoptotic and cell death rates were 47.9% and 45.7%, respectively, after HT at 43°C for 0.5 h [Figure 1A]. The expression of junction protein (ZO-1) was downregulated with an increase in temperature. When treated with HT for 0.5 and 1 h, GPX4 and TNF- α were down-regulated first and then up-regulated with the prolongation of treatment (3 and 6 h).

The cells grew well after 24 h of normal culture at 37°C. After HT treatment, most of the cells were lysed and died. After treated with cisplatin, cells shrank and developed apoptosis. To a certain extent, HT makes individual tumor cells not to cluster and can be killed easily [Figure 1B–E]. The inhibition rate was 0% and 50% in control and chemotherapy groups, respectively. The inhibition rate was raised to 67.76% after HT, while it was raised to 85.36% after thermochemotherapy at 41°C for 0.5 h. The inhibition rate reached 86.64% after HT, while it was 87.66% after thermochemotherapy at 43°C for 0.5 h. With the surgical resection of RPLS (R₀/R₁ or R₂), neoadjuvant and adjuvant therapies are applied in clinical practice to completely remove primary tumors. Cisplatin and HT, either alone or in combination, have been employed for decades to treat various malignancies. HT eliminates malignant cells by inducing hyperthermal stress. It is generally applied in combination with conventional treatments to produce more potent synergistic effects on numerous cancers.^[3] However, molecular mechanisms underlying anti-tumor effects of these therapies on cancers are not well understood, and investigation of their effects on morphology and proliferation of RPLS cells is scarce.

In the present study, we have demonstrated that 41°C is an ideal temperature for HT therapy. Notably, inhibition rate of proliferation was increased with an increase in temperature and duration of HT, with optimum temperature and duration at 41°C and for 1 h, respectively. Importantly, HT can enhance cytotoxicity of cisplatin (at 5 μ g/mL). Treatment with a combination of cisplatin and HT at 41°C was more effective at inhibiting RPLS cell proliferation than either alone. However, treatment with cisplatin and HT at 43°C failed to further improve synergistic effect. This is in agreement with previous reports that HT at 37 to 45°C shows synergistic activity with chemotherapy; however, most studies advocated HT at $\geq 42^\circ\text{C}$ with a chemotherapeutic agent such as cisplatin.^[3]

It has been demonstrated that both HT and cisplatin can induce apoptosis in various cancers.^[3] In our study, HT at 41°C for 0.5 h promoted apoptosis. When HT at 41°C was

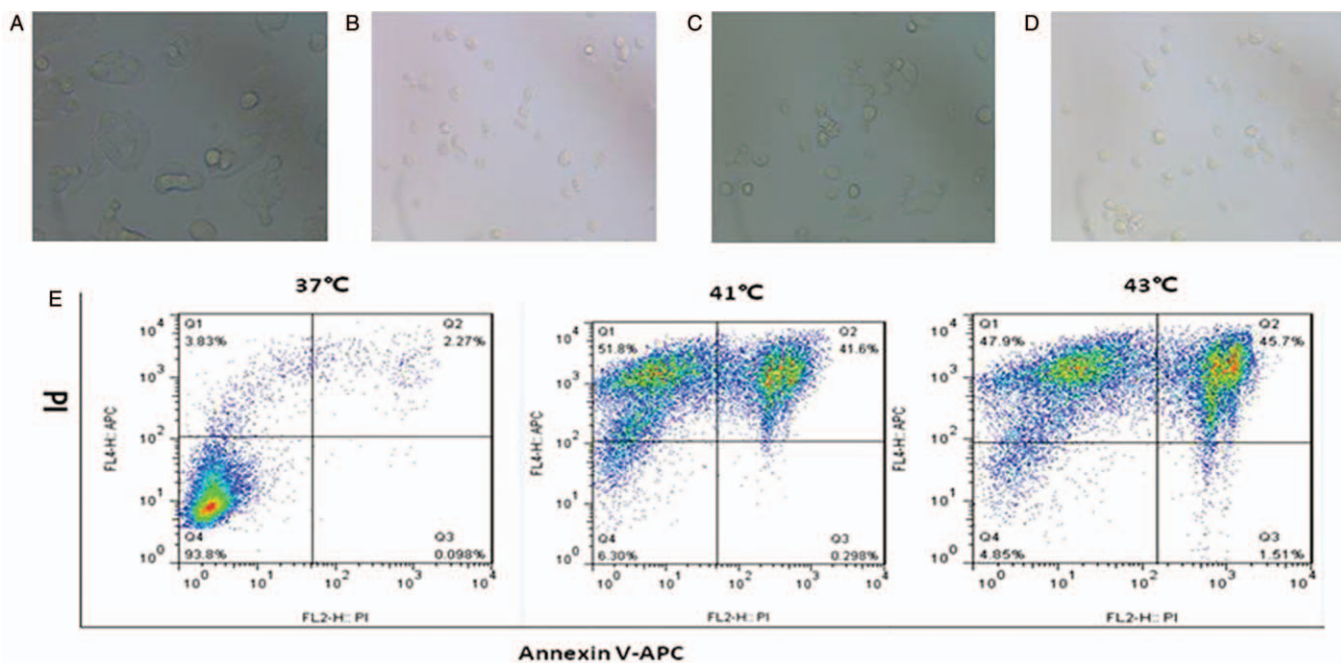


Figure 1: Flow cytometry analysis of the effects of HT on apoptosis (A), effects of HT and chemotherapy, either alone or in combination on morphology: control (B), HT (C), chemotherapy (D), and thermochemotherapy (E). HT: Hyperthermia.

applied for 30 min, 52% of SW872 cells were lysed. However, apoptotic rate reduced to 48% at 43°C. Apoptosis was obvious under microscope after HT alone, while changes in morphology were more significant when cisplatin was added to HT therapy.

HT could down-regulate ZO-1 expression. ZO-1 is a tight junction protein located in cell membrane. It mainly regulates transport of large ions between endothelium and epithelium and plays an important role in biological structure and function of tight junction.^[4] Decreased expression of ZO-1 indicates that HT can reduce adhesion between SW872 cells, and thus inhibits cell aggregation. In addition, HT upregulated GPX4 and TNF- α significantly with the prolongation of treatment. GPX4 protects normal cells against oxidative stress (lipid peroxidation). Inactivation of GPX4 results in ferroptosis. Increased expression of GPX4 is essential for cell survival in normal tissues.^[5] TNF- α belongs to TNF superfamily. It is secreted mainly by macrophages and is important for resistance to infection and transformation. Taken together, these results indicate that HT can have a strong impact on biological characteristics of SW872 cells by regulating the expression of ZO-1, GPX4, and TNF- α .

Further studies should be performed in an attempt to elucidate how RPLS cells respond to treatment with cisplatin and HT, either alone or in combination. This study has provided evidence for possible interactions of HT with ZO-1, GPX4, and TNF- α , as well as synergistic effects of cisplatin and HT on morphology and proliferation in RPLS.

Separate treatment with either cisplatin or HT can induce apoptosis in RPLS SW872 cells. HT may inhibit growth

and proliferation of SW872 cells through downregulating ZO-1 and upregulating GPX4 and TNF- α . Importantly, HT in combination with cisplatin exerts synergistic inhibitory effects on RPLS cells.

Funding

This work was supported by a grant from the Beijing Science and Technology Commission (No. Z161100000516025).

Conflicts of interest

None.

References

- Wang J, Grignol VP, Gronchi A, Luo CH, Pollock RE, Tseng WW. Surgical management of retroperitoneal sarcoma and opportunities for global collaboration. *Chin Clin Oncol* 2018;7:39. doi: 10.21037/cco.2018.07.05.
- Yang WH, Xie J, Lai ZY, Yang MD, Zhang GH, Li Y, *et al.* Radiofrequency deep hyperthermia combined with chemotherapy in the treatment of advanced non-small cell lung cancer. *Chin Med J* 2019;132:922–927. doi: 10.1097/CM9.000000000000156.
- Lee TH, Bu J, Kim BH, Poellmann MJ, Hong S, Hyun SH. Sub-lethal hyperthermia promotes epithelial-to-mesenchymal-like transition of breast cancer cells: implication of the synergy between hyperthermia and chemotherapy. *RSC Adv* 2019;9:52–57. doi: 10.1039/C8RA08472F.
- Itoh M, Nakadate K, Matsusaka T, Hunziker W, Sugimoto H. Effects of the differential expression of ZO-1 and ZO-2 on podocyte structure and function. *Genes Cells* 2018;23:546–556. doi: 10.1111/gtc.12598.
- Imai H, Matsuoka M, Kumagai T, Sakamoto T, Koumura T. Lipid peroxidation-dependent cell death regulated by GPx4 and ferroptosis. *Curr Top Microbiol Immunol* 2017;403:143–170. doi: 10.1007/82_2016_508.

How to cite this article: Miao CL, Hanif S, Zhang L, Chen XB, Huang M, Liu SB, Luo CH. Effects of cisplatin in combination with hyperthermia on biological characteristics of retroperitoneal liposarcoma. *Chin Med J* 2021;134:1110–1112. doi: 10.1097/CM9.0000000000001326