Vinpocetine enhances cisplatin sensitivity of non-small cell lung cancer cells by reducing the nuclear factor erythroid 2-related factor 2 signaling

Qingyang Zhuang,[1] Yunjian Huang,[2] Yaping Hong,[2] Wu Zhuang,[2] Kai Zhu,[2] Zhangzhou Huang [2]

[1]Department of Radiation Oncology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou, Fujian, China
[2]Department of Thoracic Oncology, Fujian Cancer Hospital & Fujian Medical University Cancer Hospital, Fuzhou, Fujian, China

Correspondence to
Zhangzhou Huang, Department of thoracic oncology, Fujian Cancer Hospital & Fujian Medical University cancer Hospital, Fuzhou, Fujian, China; huangzhangzhou@fjzlhospital.com

Accepted 13 April 2022
Published Online First 17 May 2022

To cite: Zhuang Q, Huang Y, Hong Y, *et al*. *J Investig Med* 2022;**70**:1358–1364.

ABSTRACT

Vinpocetine exerts pharmacological effects against cardiovascular diseases, while few studies focused on its roles in cancer. The present study investigated the roles of vinpocetine in non-small cell lung cancer (NSCLC) and its relationship with cisplatin resistance. A549 cisplatin-resistant cells (A549/DDP) and nuclear factor erythroid 2-related factor 2 (Nrf2)-overexpressing cell lines were established. 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay was conducted to determine cell viability. Annexin V-propidium iodide assay was conducted to determine cell apoptosis. RT-quantitative PCR and western blot analysis were conducted to determine the levels of mRNA and protein, respectively. NSCLC cell tumor-bearing model was constructed to determine the effects of vinpocetine on tumor growth. Treatment with vinpocetine inhibited cell proliferation and promoted cisplatin-induced cell apoptosis. In addition, treatment with vinpocetine suppressed protein expression of Nrf2 and inhibited messenger RNA levels of heme oxygenase 1 and NAD(P)H dehydrogenase quinone 1 induced by cisplatin. Interestingly, the overexpression of Nrf2 abolished the antiproliferative effects of vinpocetine on NSCLC cells. In vivo data suggested that vinpocetine (50 mg/kg) inhibited tumor growth and enhanced the antitumor effects of cisplatin in the NSCLC cell tumor-bearing model. Vinpocetine enhances cisplatin sensitivity of NSCLC cells in part by suppressing Nrf2 signaling.

Key messages

What is already known about this subject?
⇒ Vinpocetine exerts pharmacological effects against cardiovascular diseases, while few studies focused on its roles in cancer.

What are the new findings?
⇒ Vinpocetine enhances cisplatin sensitivity of non-small cell lung cancer (NSCLC) cells in part by suppressing nuclear factor-erythroid factor 2-related factor signaling.

How might these results change the focus of research or clinical practice?
⇒ Our study highlights the potential of vinpocetine in the treatment of NSCLC.

INTRODUCTION

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for about 85% of all lung cancer cases.[1] Cisplatin has been employed in NSCLC therapy since 1970 and is the first-line medication for the treatment of patients with NSCLC with an overall response rate of 21%.[2] In 2007, Satoh *et al* reported that cisplatin-based therapy resulted in survival rate of 19.4% and 13.9% for 2 or 3 years, respectively, in patients with advanced NSCLC.[3] Additionally, a combination of cisplatin with other medications (eg, etoposide, mitomycin C, or gemcitabine) is widely used for the treatment of NSCLC.[2 4 5] However, cisplatin resistance including inherent and acquired resistance significantly reduces the efficacy of cisplatin-based chemotherapy for the treatment of NSCLC.[6 7] Some patients with NSCLC who initially have responses to cisplatin therapy will eventually develop cisplatin resistance.[6 7] Therefore, the development of a novel strategy for NSCLC is important to overcome cisplatin resistance.

Vinpocetine is a derivative of alkaloid vincamine extracted from the Periwinkle plant and exerts broad pharmacological effects against cardiovascular diseases.[8] In 2010, Jeon *et al* reported that vinpocetine suppresses nuclear factor kappa B (NF-κB) and inflammatory mediators in a series of cell types including epithelial cells, macrophages, endothelial cells, and vascular smooth muscle cells.[9] Treatment with vinpocetine also ameliorates the mouse model of lung inflammation induced by lipopolysaccharide and tumor necrosis factor-α.[9] Rowther *et al* recently reported that treatment with vinpocetine inhibits the cell proliferation of glioblastoma multiforme cells.[10] Another study found that treatment with vinpocetine

inhibits cell proliferation of breast cancer cells by regulating cell cycle and cell apoptosis.[11] Vinpocetine also suppresses tumor growth in a breast cancer tumor-bearing animal model by the regulation of protein kinase B and signal transducer and activator of transcription factor 3.[11] However, it is still unknown whether vinpocetine exerts antitumor effects on NSCLC. Therefore, the present study investigated the effects of vinpocetine on NSCLC cell lines. Moreover, this study also explored the roles of vinpocetine in the cisplatin resistance in NSCLC.

MATERIALS AND METHODS

Cell lines and cell culture

Cell lines A549 and A549 cisplatin-resistant cells (A549/DDP) were cultured in Roswell Park Memorial Institute 1640 medium containing 10% fetal bovine serum (HyClone, Logan, Utah, USA) and 1% penicillin-streptomycin (10,000 U/mL, Gibco, Grand Island, New York, USA).

Construction of nuclear factor erythroid 2-related factor 2-overexpressing cell line

Plasmid pCDNA3-Myc3-nuclear factor erythroid 2-related factor 2 (Nrf2) was purchased from Addgene (Watertown, Massachusetts, USA). When the A549 and A549/DDP cells reached 70%–80% of confluency, the cells were transfected with pCDNA3-Myc3-Nrf2 using Lipofectamine 2000 (Thermo Fisher, Waltham, Massachusetts, USA).

Cell viability and apoptosis assay

Vinpocetine was purchased from MedChemExpress (Monmouth Junction, New Jersey, USA). Cell viability was measured by using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. In brief, the cells were seeded in the microplate and incubated for 12 hours. Next, the cells were incubated with vinpocetine or cisplatin for the indicated time. MTS solution (0.02 mL per well) was added and incubated for 2 hours. The absorbance was read under a wavelength of 490 nm using a microplate reader.

Annexin V and propidium iodide (PI) apoptosis assay was conducted to analyze cell apoptosis. In brief, after the cells were treated, cells were collected and the cell suspension was prepared. Next, annexin V-binding buffer containing annexin V was added. After that, the cells were incubated with PI staining solution in the dark. Flow cytometry (Becton, Franklin Lakes, New Jersey, USA) was conducted to analyze the populations of apoptotic cells.

Construction of tumor-bearing model and drug administration

Female BALB/c nude mice (3–4 weeks, 18–20 g) were purchased from the Animal Experimental Center (Guangdong, China). In the present study, the animals were divided into two cohorts.

In the first cohort, A549 cells were subcutaneously injected into the right flanks of mice (1×10^7 per mouse). When the tumor volume reached 100 mm^3, the mice were administrated with the vehicle, cisplatin (Cis, 2 mg/kg per 2 days; intraperitoneally), vinpocetine (Vin, 20 or 50 mg/kg; oral gavage daily), Cis (2 mg/kg per 2 days; intraperitoneally) plus Vin (20 mg/kg; oral gavage daily), or Cis (2 mg/kg per 2 days; intraperitoneally) plus Vin (50 mg/kg; oral gavage daily).

In the second cohort, A549/DDP cells were subcutaneously injected into the right flanks of mice (1×10^7 per mouse). When the tumor volume reached 100 mm^3, the mice were treated with vehicle, Cis (2 mg/kg per 2 days; intraperitoneally), Vin (20 or 50 mg/kg; oral gavage daily), Cis (2 mg/kg per 2 days; intraperitoneally) plus Vin (20 mg/kg; oral gavage daily), or Cis (2 mg/kg per 2 days; intraperitoneally) plus Vin (50 mg/kg; oral gavage daily). Tumor volume was determined every 7 days according to the formula (V=(L×W2)/2). The experimental period was 28 days. The tumor weight was recorded at the end of the experimental period.

Quantitative reverse transcription PCR

The primers were synthesized by GeneWiz (Suzhou, China). The sequence of primers is shown in the following. Heme oxygenase (*HMOX*) forward: 5'-AAG ACT GCG TTC CTG CTC AAC-3', and reverse: 5'-AAA GCC CTA CAG CAA CTG TCG-3'; NAD(P)H dehydrogenase quinone 1 (*NQO1*) forward: 5'-GAA GAG CAC TGA TCG TAC TGG C-3', and reverse: 5'-GGA TAC TGA AAG TTC GCA GGG-3'; *GAPDH* forward: 5-GGA GCG AGA TCC CTC CAA AAT-3', and reverse: 5'-GGC TGT TGT CAT ACT TCT CAT G-3'. TRIzol reagent was applied to isolate total RNA from the cells. The concentrations of RNA were then determined using a NanoDrop Spectrophotometer before reverse transcription. cDNA was synthesized before the advanced master mix was applied to quantitative analysis. To analyze the accuracy of the PCR reaction, melt curves were used. *GAPDH* was chosen as an internal control. To analyze the expressions of each target gene including *HMOX* and *NQO1*, $2^{-\triangle\triangle}$Ct values were calculated.

Western blot analysis

The primary antibodies are anticleaved caspase 3 (Proteitech, Wuhan, Hubei, China), anti-Nrf2 (Cell Signaling Technology, Shanghai, China), and anti-β-actin (Novus, Saint Charles, Missouri, USA). The secondary antibody is enzyme horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Invitrogen). Cell lysis was prepared by using radioimmunoprecipitation assay buffer supplemented with protease inhibitor. Protein concentration was determined by using a bicinchoninic acid protein kit. An equal amount (20 μg) of proteins were loaded into the sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel followed by transferring the proteins into the polyvinylidene fluoride membrane. Blocking buffer was 5% non-fat milk, which was applied before the membrane was incubated with anti-cleaved caspase 3 (1: 1000), anti-Nrf2 (1: 1000), and anti-β-actin (1: 5000) primary antibodies overnight at 4°C prior to incubating with the HRP-conjugated secondary antibodies for 2 hours at room temperature. An electrochemiluminescence detection kit was applied for chemiluminescence development. The protein expressions were normalized by the internal control GAPDH.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, California, USA). Data

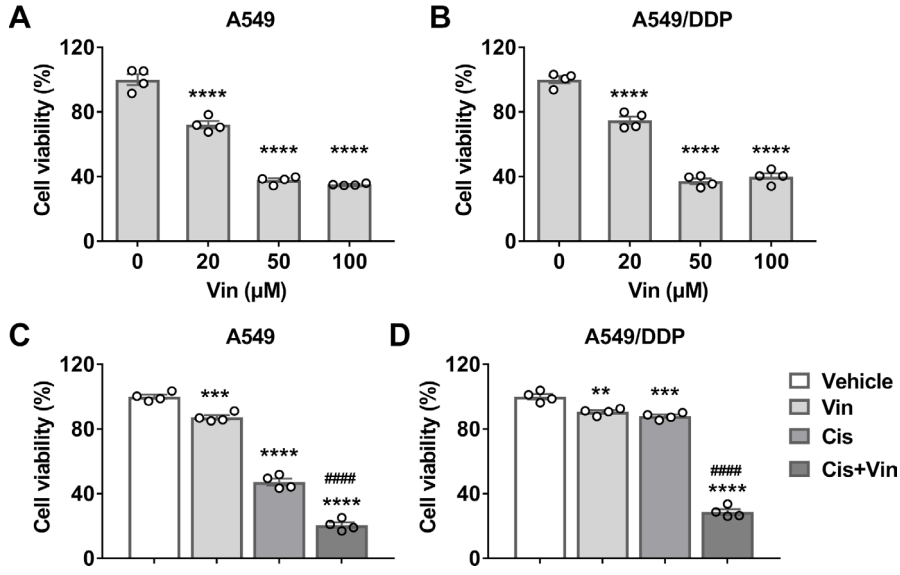

Figure 1 Vinpocetine (Vin) inhibited cell proliferation of non-small cell lung cancer cells. (A–B) The cells were treated with vinpocetine at concentrations of 0, 20, 50, or 100 μM for 72 hours. Next, cell viability was measured by using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay. (C–D) The cells were treated with vehicle or cisplatin (Cis; 5 μg/mL) with or without vinpocetine (50 μM) for 24 hours. Next, cell viability was measured by using an MTS assay. Data were shown as the means±SEM of four independent experiments. **P<0.01, ***p<0.001, ****p<0.0001, and ####p<0.0001.

were presented as the means±SEM. A two-way analysis of variance (ANOVA) test or one-way ANOVA with Tukey's multiple-comparisons test was performed. A p value <0.05 was considered statistically significant.

RESULTS

Vinpocetine inhibited cell proliferation of A549 and A549/DDP cells

We investigated the effects of vinpocetine on cell proliferation. Our results showed that treatment with vinpocetine (20–100 μM; 72 hours) significantly inhibited cell proliferation as compared with the control group (figure 1A,B). We further determined the effects of a combination of vinpocetine and cisplatin on cell proliferation. Our results showed that treatment with vinpocetine or cisplatin significantly decreased the cell viability of NSCLC cells. The inhibitory effects of vinpocetine on A549 cell proliferation were less potent than cisplatin (figure 1C,D). In addition, we also noticed that a combination of vinpocetine and cisplatin significantly decreased the cell viability of NSCLC cells. These results supported the hypothesis that vinpocetine inhibited cell proliferation and enhanced the antiproliferative effects of cisplatin on NSCLC cells.

Vinpocetine promoted cell apoptosis induced by cisplatin in A549 and A549/DDP

We further explored the effects of vinpocetine on the cisplatin-induced cell apoptosis in NSCLC cells. Our results showed that treatment with vinpocetine (50 μM) slightly increased the population of apoptotic cells (figure 2A,B). Cisplatin (5 μg/mL) significantly induced cell apoptosis in NSCLC cells, while the presence of vinpocetine further increased the population of apoptotic cells (figure 2A,B).

Furthermore, we determined the levels of apoptosis initiator, cleaved caspase-3, in NSCLC cells. Our results showed that treatment with vinpocetine (50 μM) did not affect the levels of cleaved caspase-3 in A549 and A549/DDP cells (figure 2C–F). Cisplatin-induction significantly increased the cleaved caspase-3 levels. Interestingly, we found that the presence of vinpocetine further increased the cleaved caspase-3 levels in cisplatin-induced NSCLC cells (figure 2C–F). Our results supported that treatment with vinpocetine promoted the cell apoptosis induced by cisplatin.

Vinpocetine suppressed Nrf2 signaling in NSCLC cells

Nrf2 signaling is associated with cisplatin resistance. Therefore, we investigated the effects of vinpocetine on Nrf2 signaling in NSCLC cells. Our results showed that treatment with vinpocetine (50 μM) did not affect Nrf2 protein expression in A549 cells and slightly inhibited Nrf2 protein expression in A549/DDP cells (figure 3A,B). Cisplatin-induction significantly suppressed the Nrf2 protein expression in NSCLC cells. Interestingly, the presence of vinpocetine significantly decreased Nrf2 protein expression in cisplatin-induced NSCLC cells (figure 3C,D).

We further determined the messenger RNA (mRNA) levels of Nrf2 signaling-related downstream genes including HOMX1 and NQO1 in NSCLC cells. Our results showed that treatment with vinpocetine (50 μM) decreased the mRNA levels of HOMX1 in A549 cells and did not affect the mRNA levels of HOMX1 in A549/DDP cells (figure 3E,F). However, the presence of vinpocetine significantly inhibited the mRNA levels of HOMX1 in cisplatin-induced NSCLC cells (figure 3E,F). Similarly, treatment with vinpocetine (50 μM) decreased the mRNA levels of NQO1 in NSCLC cells. And its presence further inhibited the mRNA levels of NQO1 in cisplatin-induced NSCLC cells (figure 3G,H).

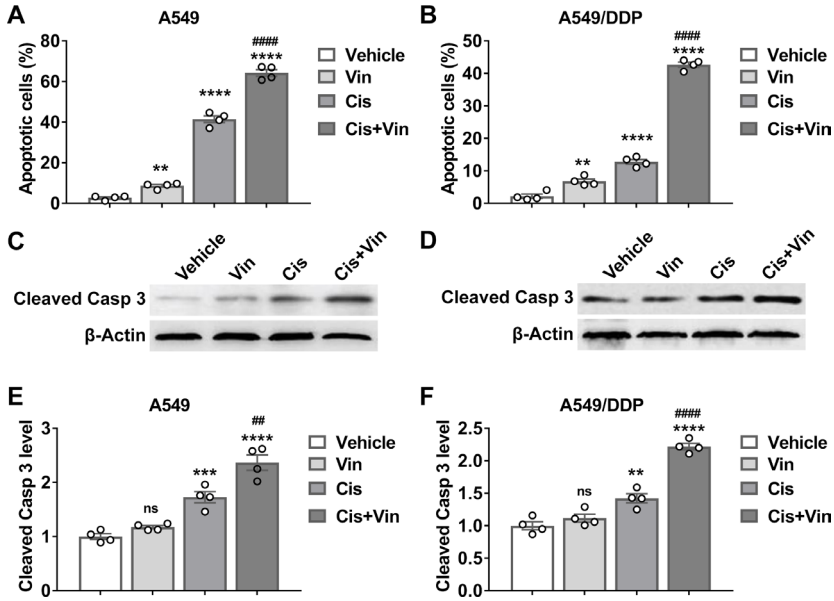

Figure 2 Vinpocetine (Vin) promoted cell apoptosis induced by cisplatin (Cis) in A549 and A549/DDP cells. (A–B) The cells were treated with vehicle or cisplatin (5 μg/mL) with or without vinpocetine (50 μM) for 24 hours. Annexin V-propidium iodide staining was performed and the population of apoptotic cells was analyzed by using a flow cytometer. (C–F) The protein levels of cleaved caspase 3 (Casp3) in the cells were determined by using western blot analysis. Data were shown as the means±SEM of four independent experiments. **P<0.01, ***p<0.001, ****p<0.0001, ##p<0.01, ####p<0.0001, and ns indicates no significance.

The overexpression of Nrf2 abolished the effects of vinpocetine on cell proliferation

To determine whether the effects of vinpocetine on the cell's sensitivity to cisplatin are associated with Nrf2, we constructed an Nrf2 overexpressing cell line. Our results showed that treatment with vinpocetine (50 μM) significantly inhibited cell proliferation of NSCLC cells (figure 4A,B). Interestingly, treatment with vinpocetine (50 μM) did not affect cell proliferation of Nrf2 overexpressed NSCLC cells (figure 4A,B). Our results suggested that the overexpression of Nrf2 abolished the effects of vinpocetine on cell proliferation.

Vinpocetine suppressed tumor growth of non-small cell lung cancer in vivo

Finally, we explored the effects of vinpocetine on the tumor growth of NSCLC in vivo. The results showed that vinpocetine (20 mg/kg) did not show significant inhibitory effects on tumor growth, while treatment with vinpocetine (50 mg/kg) significantly inhibited tumor growth in A549 tumor-bearing mice (figure 5A,B). Interestingly, the presence of vinpocetine (20 mg/kg) did not enhance the antitumor effects of cisplatin, whereas vinpocetine (50 mg/kg) significantly promoted the antitumor effects of cisplatin (figure 5A,B).

In addition, the antitumor effects of vinpocetine on A549/DDP tumor-bearing mice were also evaluated. Our results showed that vinpocetine (20 mg/kg) showed inhibitory effects on tumor volume but did not affect tumor weight. Treatment with vinpocetine (50 mg/kg) significantly suppressed tumor volume and tumor weight (figure 5C,D). Similarly, the presence of vinpocetine (20 mg/kg) did not affect the inhibitory effects of cisplatin on tumor weight but significantly suppressed the tumor volume in cisplatin-treated mice. Moreover, the presence of vinpocetine (50 mg/kg) significantly promoted the antiumor effects of cisplatin on A549/DDP tumor-bearing mice (figure 5C,D). vinpocetine suppressed tumor growth of NSCLC in vivo as well as enhanced antitumor effects of cisplatin.

DISCUSSION

In this study, we found that vinpocetine exerted antitumor effects against NSCLC in vitro and in vivo. Interestingly, we also revealed that vinpocetine enhanced cisplatin sensitivity of NSCLC cells, which was supported by the presence of vinpocetine enhanced cisplatin-induced cytotoxicity and cell apoptosis as well as antitumor effects of cisplatin in NSCLC cell tumor-bearing mice. In addition, the underlying mechanism study demonstrated that vinpocetine enhanced cisplatin sensitivity of NSCLC by the regulation of Nrf2 signaling.

Vinpocetine is known to have beneficial effects on the brain and nervous systems as well as prevent cerebrovascular disorders.[8 12 13] However, a few studies focus on the roles of vinpocetine in the treatment of cancer. To date, it is reported that vinpocetine has antitumor effects against glioblastoma multiforme and breast cancer cells.[10 11] Rowther *et al* revealed that treatment with vinpocetine inhibits cell proliferation of glioblastoma multiforme cells.[10] Another study reported that vinpocetine exerts antitumor effects against breast cancer cells in vitro and in vivo.[11] In the present study, for the first time, we found that treatment with vinpocetine was able to inhibit cell proliferation and promote cell apoptosis in NSCLC cells. Consistently, in vivo data supported that treatment with vinpocetine at a dose of 50 mg/kg/day reduced tumor growth in NSCLC cell tumor-bearing mice.

Cisplatin has been first time employed in NSCLC therapy in 1970.[14] It is the first-line medication for NSCLC therapy. However, inherent and acquired cisplatin resistance significantly reduces the efficacy of cisplatin-based chemotherapy

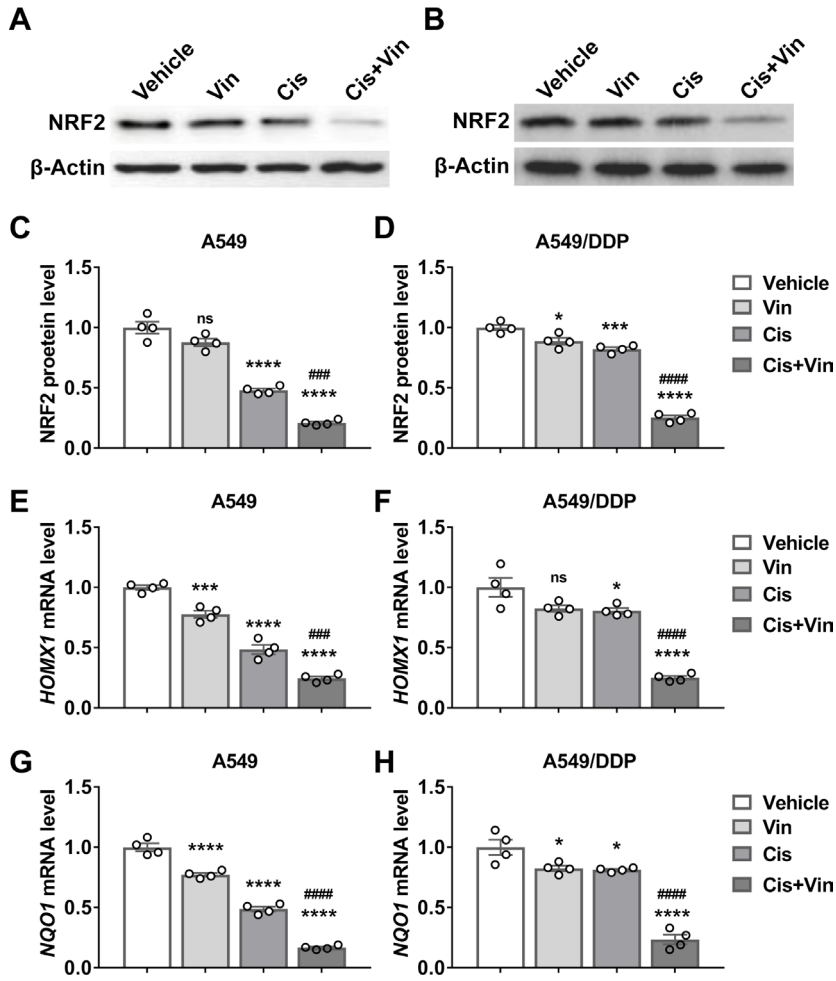

Figure 3 Vinpocetine (Vin) suppressed nuclear factor erythroid 2-related factor 2 (Nrf2) signaling in A549 and A549/DDP cells. The cells were treated with vehicle or cisplatin (Cis, 5 μg/mL) with or without vinpocetine (50 μM) for 24 hours. (A–D) The protein levels of Nrf2 in non-small cell lung cancer cells were determined by western blot analysis. (E–H) Besides, the messenger RNA (mRNA) levels of heme oxygenase 1 (HMOX1) and NAD(P)H dehydrogenase quinone 1 (NQO1) were detected by using RT-quantitative PCR. Data were shown as the means±SEM of four independent experiments. *P<0.05, ***p<0.001, ****p<0.0001, ###p<0.001, ####p<0.0001, ns indicates no significance.

in the treatment of NSCLC. Some patients with NSCLC who initially have responses to cisplatin therapy will eventually develop into cisplatin resistance.[6] It is important to develop a strategy to overcome cisplatin resistance in the treatment of NSCLC. In 2020, two previous interesting studies reported that vinpocetine reduced cisplatin-induced hepatotoxicity and acute kidney injury in rodent models.[15 16] Habib *et al* reported that treatment with vinpocetine ameliorated cisplatin-induced hepatotoxicity in mice by (1) inhibition of oxidative stress, (2) suppression of inflammatory response,

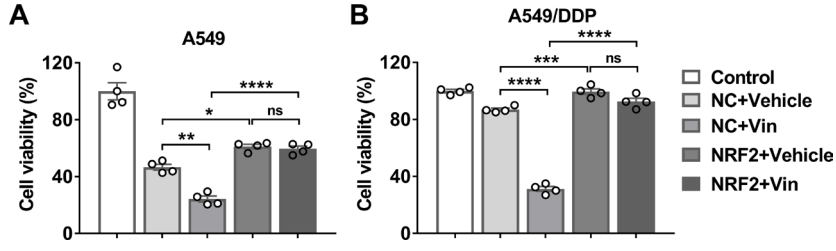

Figure 4 The overexpression of nuclear factor erythroid 2-related factor 2 (Nrf2) abolished the effects of vinpocetine (Vin) on cell proliferation. (A–B) A549 and A549/DDP cells were transfected with vector (NC) or plasmids coding Nrf2. Next, the transfected cells were treated with cisplatin (Cis, 5 μg/mL) or vehicle in the presence or absence of vinpocetine (50 μM). Cell viability was measured by using a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. Data were shown as the means±SEM of four independent experiments. *P<0.05, **p<0.01, ***p<0.001, ****p<0.0001, and ns indicates no significance.

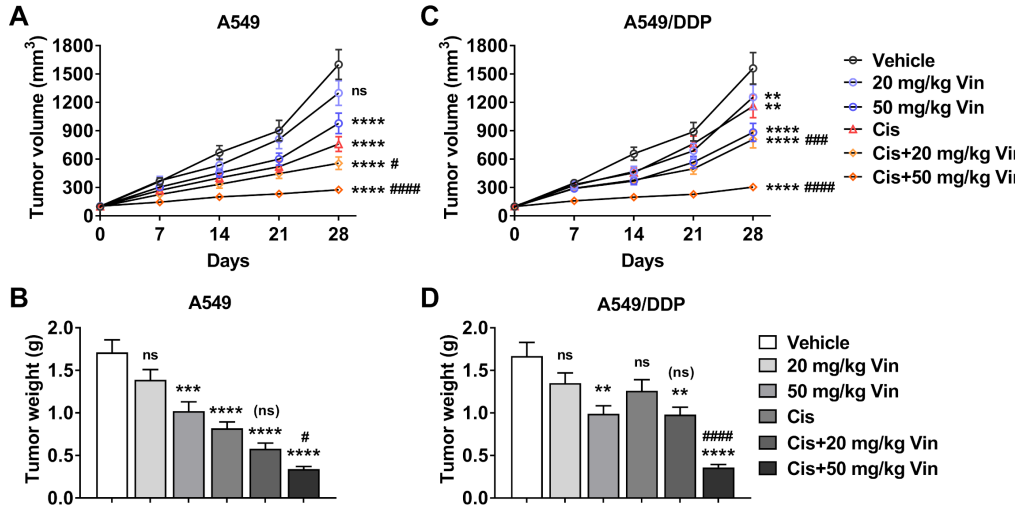

Figure 5 Vinpocetine (Vin) suppressed tumor growth in the non-small cell lung cancer cell tumor-bearing model. A549 and A549/DDP tumor-bearing mouse model was established. Next, the mice were treated vehicle or cisplatin (Cis, 2 mg/kg) with or without vinpocetine (20 or 50 mg/kg). (A–B) The tumor volume was measured every 7 days in the experimental period. (C–D) In addition, tumor weight was determined at the end of the experimental period (n=8). Data were shown as the means±SEM. **P<0.01, ***p<0.001, ****p<0.0001, $^{\#}$p<0.05, $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001, and ns indicates no significance as compared with the vehicle group and the cisplatin group.

and (3) inhibition of apoptosis.[16] Therefore, in the present study, we speculated whether vinpocetine can enhance antitumor effects in NSCLC cells. Interestingly, our result demonstrated that the presence of vinpocetine significantly promoted the cytotoxicity of cisplatin on A549 and A549/DDP cells. In addition, a combination of vinpocetine and cisplatin also induced higher populations of apoptotic cells as compared with the cisplatin-treated cells. The presence of vinpocetine also enhanced the effects of cisplatin on the cleaved caspase 3, which is consistent with a previous study, in which vinpocetine reduced serum caspase 3 in a mouse model of liver injury induced by cisplatin.[16] Moreover, treatment with vinpocetine also enhanced the antitumor effects of cisplatin in the animal tumor-bearing model, which was supported by the fact that a combination of vinpocetine and cisplatin showed stronger inhibitory effects on tumor growth as compared with the cisplatin-treated group. These results suggested that vinpocetine enhanced the antitumor effects of cisplatin on the NSCLC cell tumor-bearing model.

In addition to evaluating the effects of vinpocetine on the antitumor effects of cisplatin, we also explored whether the presence of cisplatin can reverse cisplatin resistance in NSCLC. We observed that cisplatin showed fewer toxicities on cisplatin-resistant A549/DDP cells as compared with the A549 cells. However, the presence of vinpocetine significantly enhanced the toxicities of cisplatin on the A549/DDP cells. These results supported that vinpocetine enhanced the cisplatin sensitivity of NSCLC cells.

Nrf2 is a regulator in oxidative resistance and redox signaling.[17–20] It is known that the Nrf2-mediated signaling pathway is involved in the cisplatin resistance in lung cancer cells.[21] The overexpression of Nrf2 is observed in the cisplatin-resistant cancer cells, supporting a correlation of Nrf2 and cisplatin resistance. In recent, several studies reported that Nrf2 induces cisplatin resistance by a series of mechanisms including the inhibition of iron export-related genes and regulation of cellular redox homeostasis-related proteins.[22 23] In this study, we further investigated the underlying mechanisms of vinpocetine on the regulation of cisplatin resistance. Interestingly, our results suggested that the presence of vinpocetine significantly decreased Nrf2 protein expression in cisplatin-induced NSCLC cells. Moreover, we further examined the effects of vinpocetine on Nrf2 signaling-related genes including HMOX1 and NQO1. The recruitment of Nrf2 on antioxidant response element sites increases the transcriptions of HMOX1 and NQO1.[24 25] Our results showed that the presence of vinpocetine significantly inhibited the mRNA levels of HOMX1 and NQO1 in the cisplatin-induced NSCLC cells. Moreover, to confirm whether the effects of vinpocetine on the cell's sensitivity to cisplatin are associated with Nrf2, we constructed an Nrf2 overexpressing cell line. Interestingly, our results showed that treatment with vinpocetine (50 μM) did not affect cell proliferation of Nrf2 overexpressed NSCLC cells, indicating overexpression of Nrf2 abolished the effects of vinpocetine on cell proliferation. Taken together, our results supported that vinpocetine enhanced cisplatin sensitivity of NSCLC cells by reducing Nrf2 signaling. Interestingly, in a previous study, Song *et al* reported that the effects of vinpocetine on kidney injury induced by cisplatin are in part by the regulation of Nrf2 signaling.[15] These results suggested a correlation between vinpocetine and Nrf2.

Further studies are warranted to clarify the underlying mechanisms of vinpocetine on the regulation of Nrf2 as well as its potential use in the combination therapy with cisplatin. First, it is still unclear whether vinpocetine directly binds to Nrf2 or regulates Nrf2 through other upstream genes. Further studies are warranted to explore if there is an interaction between vinpocetine and Nrf2. In addition, vinpocetine enhanced the therapeutic effects of cisplatin on NSCLC by inducing cytotoxicity and regulating apoptosis. However, to confirm the beneficial effects of vinpocetine in the cisplatin-based therapy for patients with

NSCLC, clinical trials are warranted. For instance, a combination of vinpocetine and cisplatin should be performed in the patients with NSCLC. Clinical end points including complete response, progression-free survival, and overall survival are suggested.

CONCLUSION

The presence of vinpocetine promoted cisplatin-induced cytotoxicity and cell apoptosis in NSCLC cells. The effects of vinpocetine on cisplatin sensitivity were associated with Nrf2 signaling as Nrf2 overexpressing abolished the antiproliferative effects of vinpocetine. In vivo data also suggested that the presence of vinpocetine (50 mg/kg) enhanced the antitumor effects of cisplatin. Taken together, vinpocetine enhanced the cisplatin sensitivity of NSCLC cells by reducing Nrf2 signaling.

Contributors Data curation, analysis: QZ, YHu, YHo, WZ, KZ; drafting of the manuscript: QZ, YHu, YHo, WZ, KZ, ZH; concept, design of the study: QZ, ZH. guarantor: ZH. All authors approved the publication of the manuscript.

Funding This research was supported by the Natural Science Foundation of Fujian (2018J01274).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The standard animal procedures were reviewed and approved by Fujian Cancer Hospital (#2021.03.007c).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

ORCID iD

Zhangzhou Huang http://orcid.org/0000-0002-6639-3607

REFERENCES

1 Goldstraw P, Ball D, Jett JR, *et al*. Non-small-cell lung cancer. *Lancet* 2011;378:1727–40.
2 Arriagada R, Bergman B, Dunant A, *et al*. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med* 2004;350:351–60.
3 Satoh H, Ishikawa H, Ohara G, *et al*. Long-term survivors after chemotherapy in advanced non-small cell lung cancer. *Anticancer Res* 2007;27:4457–60.
4 Schaake-Koning C, van den Bogaert W, Dalesio O, *et al*. Effects of concomitant cisplatin and radiotherapy on inoperable non-small-cell lung cancer. *N Engl J Med* 1992;326:524–30.
5 Winton T, Livingston R, Johnson D, *et al*. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. *N Engl J Med* 2005;352:2589–97.
6 Sarin N, Engel F, Kalayda GV, *et al*. Cisplatin resistance in non-small cell lung cancer cells is associated with an abrogation of cisplatin-induced G2/M cell cycle arrest. *PLoS One* 2017;12:e0181081.
7 Rosell R, Lord RVN, Taron M, *et al*. DNA repair and cisplatin resistance in non-small-cell lung cancer. *Lung Cancer* 2002;38:217–27.
8 Patyar S, Prakash A, Modi M, *et al*. Role of vinpocetine in cerebrovascular diseases. *Pharmacol Rep* 2011;63:618–28.
9 Jeon K-I, Xu X, Aizawa T, *et al*. Vinpocetine inhibits NF-kappaB-dependent inflammation via an IKK-dependent but PDE-independent mechanism. *Proc Natl Acad Sci U S A* 2010;107:9795–800.
10 Rowther FB, Wei W, Dawson TP, *et al*. Cyclic nucleotide phosphodiesterase-1C (PDE1C) drives cell proliferation, migration and invasion in glioblastoma multiforme cells in vitro. *Mol Carcinog* 2016;55:268–79.
11 Huang E-W, Xue S-J, Zhang Z, *et al*. Vinpocetine inhibits breast cancer cells growth in vitro and in vivo. *Apoptosis* 2012;17:1120–30.
12 Szatmari SZ, Whitehouse PJ. Vinpocetine for cognitive impairment and dementia. *Cochrane Database Syst Rev* 2003:CD003119.
13 Hagiwara M, Endo T, Hidaka H. Effects of vinpocetine on cyclic nucleotide metabolism in vascular smooth muscle. *Biochem Pharmacol* 1984;33:453–7.
14 Fennell DA, Summers Y, Cadranel J, *et al*. Cisplatin in the modern era: the backbone of first-line chemotherapy for non-small cell lung cancer. *Cancer Treat Rev* 2016;44:42–50.
15 Song W, Yin W, Ding L, *et al*. Vinpocetine reduces cisplatin-induced acute kidney injury through inhibition of NF-κB pathway and activation of Nrf2/ARE pathway in rats. *Int Urol Nephrol* 2020;52:1389–401.
16 Habib SA, Abdelrahman RS, Abdel Rahim M, *et al*. Anti-apoptotic effect of vinpocetine on cisplatin-induced hepatotoxicity in mice: the role of annexin-V, caspase-3, and Bax. *J Biochem Mol Toxicol* 2020;34:e22555.
17 Jaramillo MC, Zhang DD. The emerging role of the Nrf2-Keap1 signaling pathway in cancer. *Genes Dev* 2013;27:2179–91.
18 Ma Q. Role of nrf2 in oxidative stress and toxicity. *Annu Rev Pharmacol Toxicol* 2013;53:401–26.
19 Liu C, Guo H, DaSilva NA, *et al*. Pomegranate (*Punica granatum*) phenolics ameliorate hydrogen peroxide-induced oxidative stress and cytotoxicity in human keratinocytes. *J Funct Foods* 2019;54:559–67.
20 Liu C, Guo H, Dain JA, *et al*. Cytoprotective effects of a proprietary red maple leaf extract and its major polyphenol, ginnalin a, against hydrogen peroxide and methylglyoxal induced oxidative stress in human keratinocytes. *Food Funct* 2020;11:5105–14.
21 Silva MM, Rocha CRR, Kinker GS. The balance between NRF2/GSH antioxidant mediated pathway and DNA repair modulates cisplatin resistance in lung cancer cells. *Sci Rep* 2019;9:1–11.
22 Bao L-J, Jaramillo MC, Zhang Z-B, *et al*. Nrf2 induces cisplatin resistance through activation of autophagy in ovarian carcinoma. *Int J Clin Exp Pathol* 2014;7:1502.
23 Bao L, Wu J, Dodson M, *et al*. ABCF2, an Nrf2 target gene, contributes to cisplatin resistance in ovarian cancer cells. *Mol Carcinog* 2017;56:1543–53.
24 Sarutipaiboon I, Settasatian N, Komanasin N, *et al*. Association of genetic variations in NRF2, NQO1, HMOX1, and MT with severity of coronary artery disease and related risk factors. *Cardiovasc Toxicol* 2020;20:176–89.
25 Johnson GS, Li J, Beaver LM, *et al*. A functional pseudogene, NMRAL2P, is regulated by Nrf2 and serves as a coactivator of NQO1 in sulforaphane-treated colon cancer cells. *Mol Nutr Food Res* 2017;61:1600769.