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Gemcitabine combined with baicalein exerts antiviral activity against PEDV by inhibiting the entry and replication phases

Yingge Zheng[1,2,3], Yanfei Zhang[1,2,4], Fangying Xiong[1,2,5], Dehua Luo[1,2,5], Yuxiang Wang[1,2,4,5], Yijia Zhang[1,2] and Dengguo Wei[1,2,3,4,5*]

Abstract

Cocktail therapy significantly reduces the development of resistance to individual medications due to viral mutations. However, for effective inhibition of a particular virus, a customized approach to combination pharmacotherapy may be essential. Porcine epidemic diarrhea virus (PEDV) is a member of the Coronaviridae family, whose genome consists of a single strand of positive-sense RNA and has evolved into multiple epidemic lineages with no available drugs in clinical practice. In this study, we found that the nucleoside analog gemcitabine decreased the titer of PEDV, with a median effective concentration (EC_{50}) of 3.12 μM, thereby inhibiting viral replication. The natural product baicalein acts by targeting the early entry stage and directly inactivates the virus, with an EC_{50} of 5.02 μM. A notable synergistic effect was observed with the combination of 1 μM gemcitabine and 1.5 μM baicalein. This study demonstrated that the strategic use of a drug combination targeting both the replication and entry phases of the PEDV lifecycle can effectively inhibit viral proliferation.

Keywords Antivirus, Gemcitabine, Baicalein, Synergistic effect, Combination therapy

Introduction

The concurrent administration of two or more drugs, each targeting different proteins involved in the viral replication cycle, can act synergistically to inhibit viral growth, as exemplified by the use of combination therapy, or "cocktail therapy," for HIV control. This approach not only reduces the dosage of individual drugs but also mitigates the development of resistance associated with prolonged use of single-target medications (Lopez and Banerji 2016). Another common combination therapy was the treatment of COVID-19. While 80% of SARS-CoV-2 replication can be inhibited with the concentration of 20.18 μM remdesivir, a similar level of inhibition is achieved with only 5 μM remdesivir, combined with omipalisib (Jang et al. 2021). Therefore, the pursuit of combination drug therapies remains a critical strategy in the development of antiviral treatments(Shyr et al. 2021; Sun et al. 2016; White et al. 2021).

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*Correspondence:
Dengguo Wei
dgwei@mail.hzau.edu.cn
[1] State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei, China
[2] Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan 430070, Hubei, China
[3] College of Veterinary Medicine, National Reference Laboratory of Veterinary Drug Residues (HZAU) and MAO Key Laboratory for Detection of Veterinary Drug Residues, Huazhong Agricultural University, Wuhan 430070, Hubei, China
[4] Shenzhen Institute of Nutrition and Health, Huazhong Agricultural University, Shenzhen 518000, China
[5] Shenzhen Branch, Genome Analysis Laboratory of the Ministry, Huazhong Agricultural University, Shenzhen 518000, China

Viruses employ various strategies to proliferate within hosts, which calls for the development of targeted combination therapy approaches for specific viruses. The concurrent use of the HCV protease inhibitors SCH503034 and Clemizole, an inhibitor of NS4B RNA, has been proved to potently inhibit HCV replication through synergistic effects while also reducing the emergence of drug resistance and hindering cross-resistance (Einav et al. 2010). In another example, the combined application of Molnupiravir, which targets the replication of SARS-CoV-2, along with HTA, a small molecule that interacts with TMPRSS2, has a synergistic effect on the inhibition of SARS-CoV-2 infection. Compared with monotherapy, this combination therapy results in a 2-3 three fold reduction in the required drug concentration (Wagoner et al. 2022).

As an alpha coronavirus, PEDV typically affects piglets within the first week of life, making it challenging to control PEDV through active immunization of young animals (Lee 2015; Zhang et al. 2022). The passive immunity conferred by immunizing sows to safeguard newborn piglets is influencd by various factors, resulting in suboptimal clinical control (Zhang et al. 2022). The development of environmentally sustainable novel pharmaceuticals targeting porcine intestinal coronaviruses constitutes a crucial approach to assist neonatal piglets in undergoing the perilous early stage of life, thereby effectively reducing the incidence of diarrhea and mortality rates (Yang et al. 2023). Over the last decade, several targeted treatment have been developed such as ZINC12899676, a natural compound, which inhibits PEDV proliferation by targeting viral NTPase (Wang et al. 2022), and PA-824, which significantly inhibits PEDV proliferation by inhibiting apoptosis (Li et al. 2024). Nonetheless, despite these advancements, the therapeutic efficacy of these compounds is not yet sufficient to meet clinical demands, highlighting the urgent need for the discovery of new antiviral agents or innovative treatment approaches.

This study identified gemcitabine, a nucleoside analog, as an effective anti-PEDV agent that targets the viral replication phase. The naturally occurring compound baicalein is capable of directly inactivating PEDV particles and suppressing the virus's early entry into host cells. When used in combination, these two compounds exhibit a synergistic effect on the inhibition of PEDV proliferation. The most significant synergy was observed with 1 μM gemcitabine and 1.5 μM baicalein. Research has underscored the therapeutic potential of using compounds that target different stages of PEDV proliferation, including entry and replication, to increase anti-PEDV efficacy.

Results

Gemcitabine inhibits the proliferation of PEDV

On the basis of the potential of nucleoside analogs in antiviral therapy, we collected four unreported nucleotide analogs: gemcitabine, 8-azaguanine, cytidine, and deoxycytidylate to test their antiviral activity against PEDV (Fig. 1A). The IFA results revealed that the Gemcitabine-treated group, in contrast to the DMSO group, had nearly no lesions. However, the groups treated with 8-Azaguanine, Cytidine, and Deoxycytidylate suffered from severe lesions (Fig. 1B). The $TCID_{50}$ results revealed no appreciable significant difference in virus titers between the cytidine-treated groups and the DMSO-treated groups while the 8-azaguanine- and gemcitabine- and deoxycytidylate-treated groups exhibited significantly lower PEDV viral titers. The gemcitabine-treated group presented a reduction in the viral titer of ~5.8 log10 (Fig. 1C). These results indicate that gemcitabine has the most significant antiviral activity against PEDV among these compounds.

Gemcitabine exerts antiviral activity in the replication phase

To assess the safety profile of gemcitabine, a CCK-8 cytotoxicity assay was performed. The concentration of gemcitabine was diluted from 200 μM to 6.25 μM, and the results revealed that the CC_{50} (50% cytotoxicity) of gemcitabine was > 200 μM (Fig. 2A). To investigate the antiviral effects of gemcitabine on PEDV, cells were exposed to various concentrations of gemcitabine following infection with PEDV. The lesions were evaluated by IFA, which revealed a notable difference in the number of infected cells between the gemcitabine-treated group and the DMSO group (see Supplementary Figure S1A). Compared with that of the DMSO group, the expression of the PEDV N protein was undetectable by IFA at a concentration of 10 μM, which indicated that gemcitabine exhibited a good inhibitory effect on PEDV. The analysis of $TCID_{50}$ and qRT−PCR results revealed a dose-dependent decrease in response to the different concentrations of the compounds (Fig. 2B and D). Compared with that in the DMSO group, the virus titer in the 10 μM gemcitabine treatment group was reduced by ~4 log10 (Fig. 2B). The EC_{50} of gemcitabine was calculated to be 3.12 μM (Fig. 2C). These results consistently indicated that gemcitabine exhibited antiviral activity against PEDV in a concentration-dependent manner.

To investigate how different stages of PEDV infection are influenced by gemcitabine, different concentrations of gemcitabine (5 μM, 10 μM) were used to assess their effects on each stage of PEDV infection. As shown in Fig. 2E, Gemcitabine treatment directly inhibited the replication phase of PEDV. The virus titer of the gemcitabine-treated group was associated with the prevalence

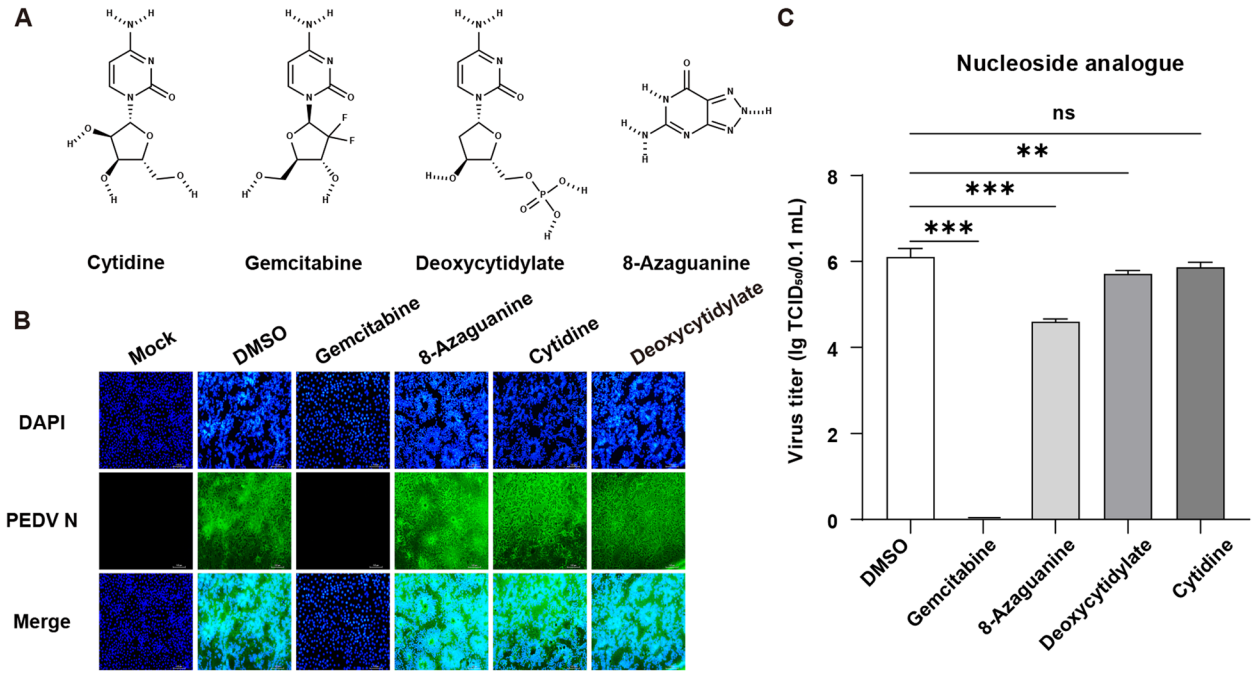

Fig. 1 Inhibitory effects of nucleoside analogs on PEDV infection. **A** The structure of nucleoside analogs. **B** The antiviral effects of nucleoside analogs were evaluated via indirect immunofluorescence. Microscope scale: 100 μm. IFA images showing the viral N protein in green and nuclei in blue. The experiments were performed three times and from 1 random horizon in each experiment. **C** Vero cells were infected with PEDV at 0.1 MOI in the presence of the nucleoside analogs at 20 μM for 18 h, and the virus titer was measured via a $TCID_{50}$ assay. The data were expressed as the means and standard deviations from three independent experiments and were analyzed via one-way ANOVA. ns, $p > 0.05$; ***, $p < 0.001$

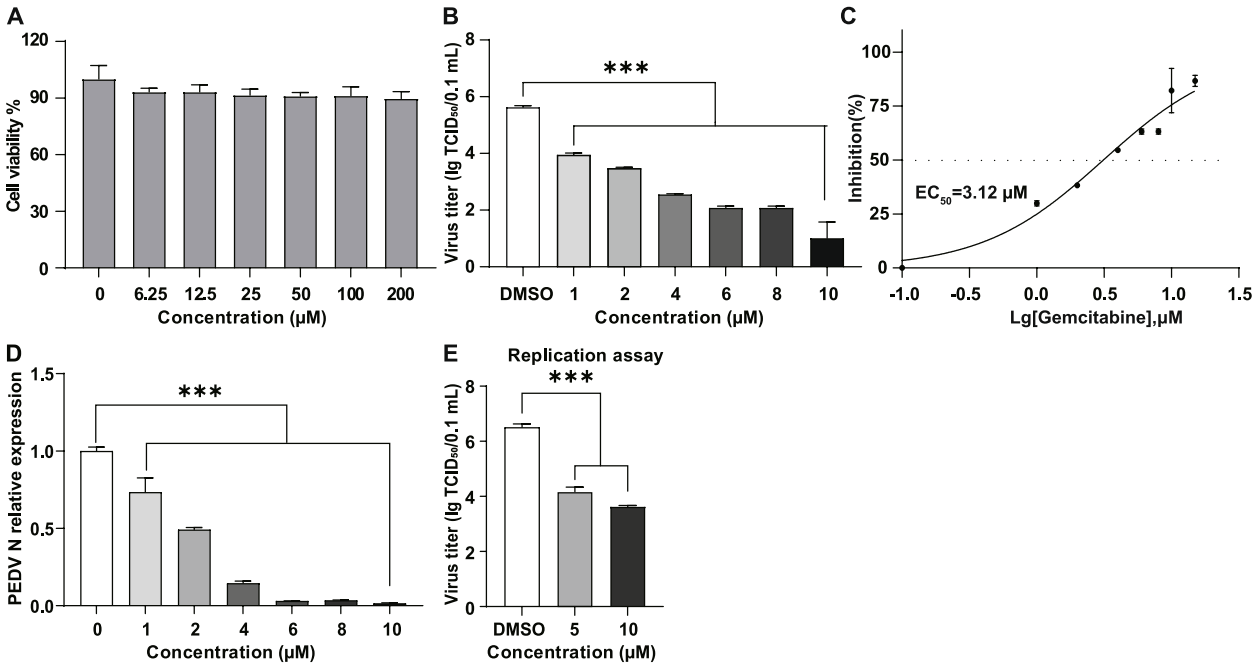

Fig. 2 Gemcitabine inhibited PEDV proliferation in a dose-dependent manner. **A** Cell viability was estimated by a CCK-8 assay. **B** Culture lysates were collected for virus titration. The results are expressed as the $TCID_{50}$. **C** EC_{50} values were calculated via GraphPad Prism 8.0. **D** The effects of gemcitabine on PEDV N gene expression were detected via qRT-PCR. **E** The effect of gemcitabine on the replication phase of PEDV proliferation was assessed via a $TCID_{50}$ assay. The data were expressed as the means and standard deviations from three independent experiments and were analyzed via one-way ANOVA. ***, $p < 0.001$

of viral plaques. A notable decrease in the virus titer was detected in the gemcitabine group. Compared with those in the DMSO group, the virus titer in the 10 μM gemcitabine treatment group was ~ 2.4 log10 lower (Fig. 2E). To evaluate the influence of gemcitabine on different stages of PEDV infection, a $TCID_{50}$ assay was carried out. The findings revealed that the virus titers of the groups treated with different concentrations of gemcitabine were nearly identical to those of the DMSO group. An additional figure provides a more detailed depiction (see Supplementary Figure S1B-D). Taken together, these results suggest that the antiviral efficacy of gemcitabine against PEDV occurs primarily through disruption of the viral replication phase.

Baicalein exerts anti-PEDV activity by inhibiting the early stages of PEDV proliferation

To explore the anti-PEDV activity of natural products, cytopathic effects (CPEs) were observed in eight natural products: baicalein, chlorogenate, baicalin, precose, harringtonin, palmatine, paeoniflorin, quercetin groups, and the DMSO group, including cell fusion and massive virion (see Supplementary Figure S2A). The baicalein groups did not present any symptoms of CPE. The $TCID_{50}$ assay revealed that, in contrast to the DMSO treatment group, the groups treated with chlorogenate, baicalin, precose, harringtonin, palmatine, paeoniflorin, or quercetin did not significantly inhibit viral plaques. The baicalein treatment group presented a reduction in the virus titer of ~ 4.8 log10 (see Supplementary Figure S2B). These results suggested that baicalein exhibited significant anti-PEDV activity at a concentration of 20 μM. To evaluate the cytotoxicity of baicalein, a range of concentrations from 0.2 μM to 200 μM was used for subsequent cytotoxicity evaluation. The CC_{50} of baicalein determined by the CCK-8 assay was greater than 200 μM (Fig. 3B and S3A). The cell viability of the baicalein treatment group was greater than 80% at a concentration of 50 μM. The results of the IFA and $TCID_{50}$ experiments indicated that baicalein inhibited PEDV proliferation in a dose-dependent manner (Fig. 3B) with more details shown in additional figure (see Supplementary Figure S3A). The EC_{50} of baicalein was calculated as 5.02 μM. PEDV N mRNA expression was substantially decreased in the baicalein-treated group (Fig. 3D and E).

To determine the specific stage of virus proliferation affected by baicalein, baicalein (5 μM, 10 μM) was added to the cell culture medium of Vero cells under various

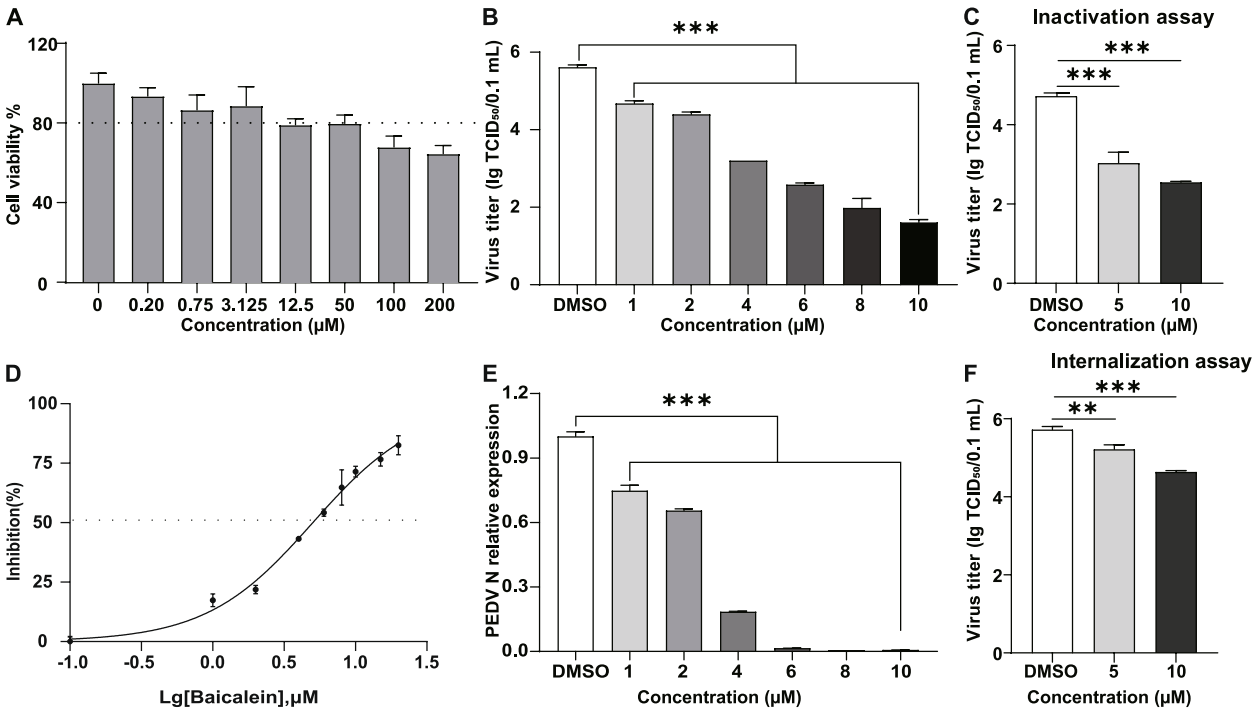

Fig. 3 Baicalein significantly restricts PEDV infection. Vero cells were incubated with baicalein (5,10 μM) and PEDV (MOI = 0.1) as described in the Methods section. **A** The viability of baicalein-treated cells was estimated via a CCK-8 assay. **B** Culture lysates were collected for virus titration. The results are expressed as the $TCID_{50}$. **C** The effect of baicalein on the inactivation of PEDV proliferation was assessed via a $TCID_{50}$ assay. **D** EC_{50} values were calculated via GraphPad Prism 8.0. **E** The effects of baicalein on PEDV N gene expression were detected via qRT–PCR. **F** The effect of baicalein on the internalization phase of PEDV proliferation was assessed via a $TCID_{50}$ assay. The data are expressed as the means and standard deviations from three independent experiments and were analyzed via one-way ANOVA. **, $0.001 \le p < 0.01$; ***, $p < 0.001$

conditions, after which the virus titer was measured. The $TCID_{50}$ results from the inactivation stage assay indicated that the virus titer in the 10 μM baicalein treatment group was reduced by ~ 2 log10 compared with that in the DMSO group (Fig. 3C), indicating a direct inactivating effect on PEDV of baicalein. The results of the internalization stage indicated that the virus titer decreased in the 10 μM treatment group (Fig. 3F). In the other stages, no significant difference in virus titer was detected between the baicalein-treated group and the DMSO-treated group (see Supplementary Figure S3B-D). In summary, baicalin primarily inhibited the early entry phases of the virus and directly inactivated the virus to exert an antiviral effect.

Gemcitabine synergizes with baicalein to inhibit PEDV proliferation

Gemcitabine affected the replication stage of PEDV proliferation, whereas baicalein inhibited the early entry stage and directly inactivated the virus. Considering their distinct modes of action, we used the compounds in combination to explore whether synergistic effects exist in the inhibition of PEDV proliferation. The concentrations of the combinations were established based on the EC_{50} values and the cell viability of the compounds was ascertained. Then, the compounds were cross-combined and added into the cell culture medium at varying concentrations for a 24-h incubation period to assess cell viability. The results of the CCK-8 assay indicated that cell viability exceeded 80% (Fig. 4A), indicating that the combined drug did not have toxic effects on Vero cells. The compounds were subsequently combined and incubated with 0.1 MOI PEDV to explore the inhibitory effects. As shown in Fig. 4E, the relative level of the PEDV N protein gradually decreased with increasing combined concentration (Fig. 4A). The $TCID_{50}$ results indicated that, compared with those in the single drug treatment groups, the virus titers in the combined treatment groups were considerably lower. Compared with the DMSO treatment, the combination of 0.75 μM baicalein and 0.5 μM gemcitabine reduced the virus titer by ~ 2.5 log10 (Fig. 4B), while it could be reduced by 13.18-fold with 1 μM gemcitabine and 22.39-fold with 1.5 μM baicalein. Nevertheless, the combination of these two compounds exceedingly reduced the virus titer by 6.76×10^3-fold with a 14.4 mean synergistic score, indicating a robust synergistic effect between gemcitabine and baicalin (Ianevski et al. 2020). The optimal synergistic effect was observed when 1 μM gemcitabine was combined with 1.5 μM baicalein (Fig. 4C and D). These data confirmed that the combination of gemcitabine and baicalein could reduce the required concentrations and exert significant anti-PEDV activity.

Discussion

As an RNA virus, PEDV has a high mutation rate and has diversified into multiple epidemic lineages(Mei et al. 2023). However, specific antiviral drugs have not yet been developed. Combination therapy has emerged as an effective strategy to combat drug resistance caused by mutations. This study demonstrated that the nucleoside analog gemcitabine inhibits the replication of PEDV. The selectivity index of gemcitabine was greater than 50, and the EC_{50} was 3.12 μM. Additionally, gemcitabine can inhibit SARS-CoV-2 proliferation by regulating nucleotide biosynthesis (Zhang et al. 2020). Nevertheless, further experimental validation is needed to ascertain whether gemcitabine targets the RNA-dependent RNA polymerase of PEDV. Baicalein, a natural compound predominantly derived from Scutellaria baicalensis, effectively inhibits PEDV entry and directly inactivates the virus, resulting in an EC_{50} of 5.02 μM and a selectivity index exceeding 33. The two compounds have different mechanisms of action, which was demonstrated to enhance antiviral efficacy at reduced dosages when administered in combination. The synergistic results from SynergyFinder indicated that the combination of gemcitabine and baicalein can exert synergistic effects at different concentrations (Ianevski et al. 2020). The inhibitory effect of the best combination was more than 300 times greater than that of the single compounds.

Combination therapy has been employed for over three decades as a strategy to enhance efficacy and diminish toxicity, showing promising potential in the field of antiviral therapy (Anighoro et al. 2014). However, how to combine different types of inhibitors to achieve maximum efficacy is a thought-provoking question. Repurposing drugs for combined utilization represents a praiseworthy concept, which resumed after the outbreak of COVID-19 (Luo et al. 2022). The repurposed drug possesses the advantages of excellent safety and is highly suitable for utilization as a combined drug (Riva et al. 2020). Natural products have become mainstream in antiviral drug research because of their diverse sources and diverse mechanisms (Chen et al. 2020; Yi et al. 2020; Yu et al. 2018). Gemcitabine, a commercialized drug, was repurposed as an antiviral drug in this study and demonstrated to have synergistic efficacy in combination with the natural product baicalein (Dasari et al. 2022; van der Heijden et al. 2023). Gemcitabine may be a promising safe candidate for future animal experiments against PEDV (Barton-Burke 1999). The synergistic effect of gemcitabine and baicalein on the inhibition of PEDV infection will be examined in live animals to validate their combined efficacy in vivo, which could pave the way for a novel antiviral therapeutic strategy. In addition, antiviral combination therapies, such as biotherapy combined

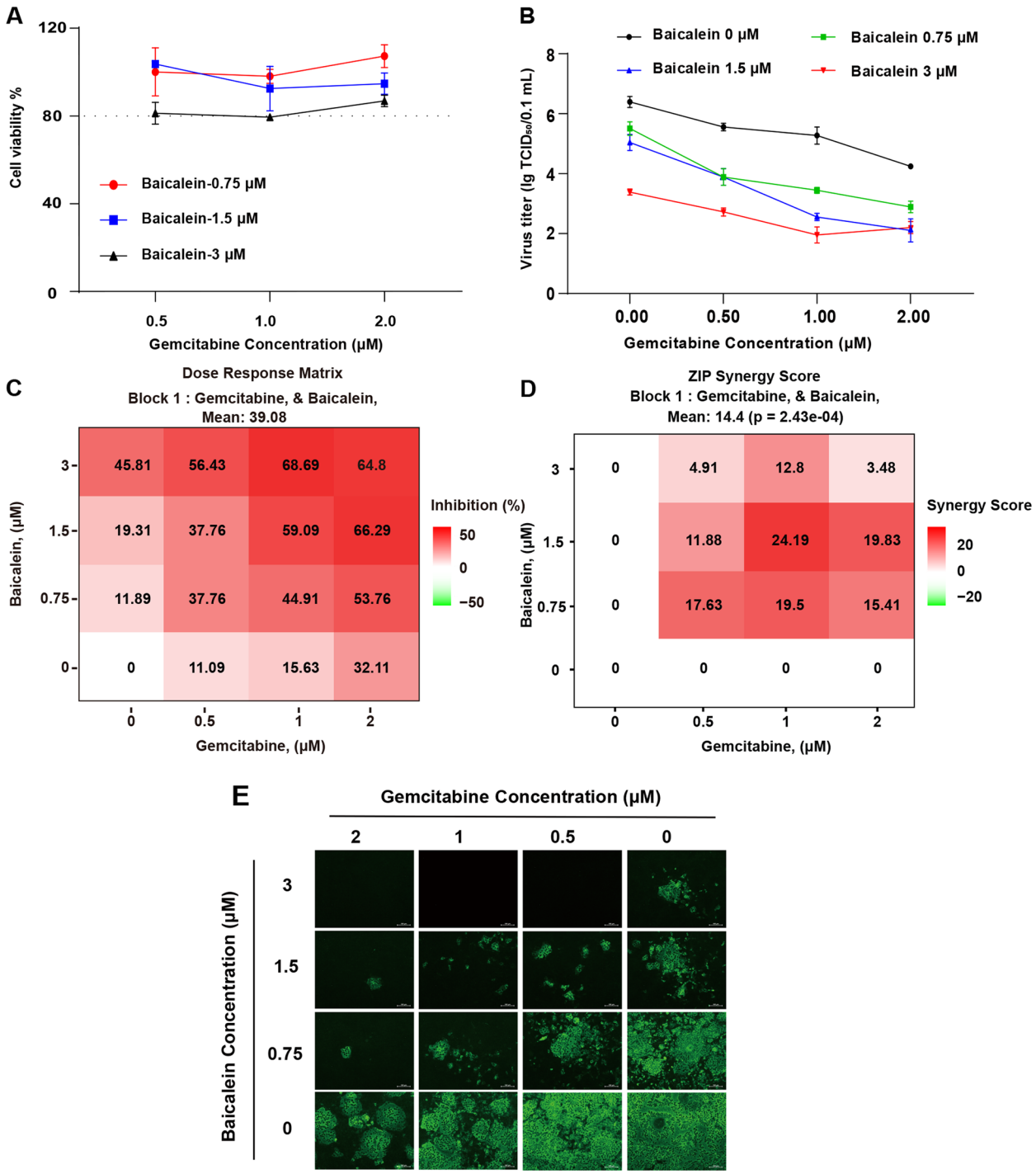

Fig. 4 Gemcitabine synergizes with baicalein to inhibit PEDV proliferation. **A** A CCK-8 assay was performed to detect the viability of cells treated with different concentrations of the combination of gemcitabine and baicalein. **B** $TCID_{50}$ assay of the effects of the combination of gemcitabine and baicalein on the PEDV titer. **C** Evaluation of the effects of the combination of gemcitabine and baicalein on PEDV. **D** ZIP model evaluation of the combined effect of baicalein and gemcitabine. The synergetic volume regions are highlighted in red. The mean synergy score for a drug combination was averaged over all the dose combination measurements. **E** Effects of the combination of gemcitabine and baicalein on PEDV N protein expression, as measured by IFA. Green light represents the expression of the PEDV N protein

with small molecules, antibody–drug conjugates, and small molecules combined with nucleic acid drugs, have gradually developed in recent years (Bojkova et al. 2022; Ianevski et al. 2021; Li et al. 2020; Mendoza et al. 2018; Wei et al. 2024). These studies highlight the potential of combination therapy in the antiviral field. However, research on anti-PEDV drugs has focused mostly on individual drugs. Compared with monotherapy, combination therapy against PEDV infection can reduce the dosage of drugs used and enhance their efficacy, which was proved to be a promising therapeutic approach.

Conclusions

It has been demonstrated that gemcitabine and baicalein inhibit the proliferation of PEDV through different mechanisms. The combination of these two compounds has a synergistic effect. The outcomes of this study provide valuable contributions to the advancement of therapeutic regimens, specifically in the realm of combination therapy and the formulation of robust antiviral strategies targeting PEDV.

Methods

Cells, viruses and compounds

Vero cells were obtained from ATCC, and the culture medium was DMEM (Gibco) supplemented with 10% FBS at 37°C and 5% CO_2. The PEDV AJ1102 strain and the antibodies against PEDV N were provided by Professor Xiao Shaobo from Huazhong Agricultural University. The compounds obtained from Macklin were prepared as solutions in DMSO (dimethyl sulfoxide).

Indirect immunofluorescence assay (IFA)

Vero cells were cultured on plates. The test compounds were added at various concentrations and cultured for 2 h while the cells were 80% confluent. The cell culture medium was subsequently replaced with PEDV infection for 2 h, following by a re-exposure to the same concentrations of the test compounds for 18 h. Afterwards, the cells were fixed and subsequently treated with precooled methanol after being washed with PBS. The washing mixture was then incubated with 5% BSA for 2 h. After that, the cells were treated with a monoclonal antibody against the PEDV N protein (1:500 diluted with PBS) and exposed to an Alexa 488-conjugated anti-mouse secondary antibody (Antgene, Wuhan, China). For nuclear visualization, 4′,6-diamidino-2-phenylindole (DAPI) (Beyotime Biotechnology, Shanghai, China) staining was performed for 15 min. The IFA results were examined via electrokinetic fluorescence microscopy (Olympus). The scale of the microscope was 100 μm.

RNA extraction and quantitative real-time PCR (qRT–PCR)

Total RNA was reverse transcribed to cDNA, and two-step RT-PCR was carried out. The data from the compound-treated groups were analyzed relative to those from the untreated group via the $2^{-\Delta\Delta CT}$ approach. Each mRNA sample was analyzed for three independent measurements. The specific primers used in the assay were as follows:

qβ-actin-F: 5'-CCACCATGTACCCTGGCATT-3';
qβ-actin-R: 5'-ACTCCTGCTTGCTGATCCAC-3';
qPEDV-F: 5'-CTGGGTTGCTAAAGAAGGCG-3';
qPEDV-R: 5'-CTCGGGAGCTGTTGAGAGAA-3'.

Cytotoxicity assay

When the monolayers of cells in the 96-well plate were cultured to 80% confluence, the medium was replaced with media containing compounds at concentrations ranging from 0 to 200 μM. The cells were incubated for 24 h (Li et al. 2024). A control group of cells was cultured with medium without compounds. After 24 h, the cytotoxicity of the compounds was reflected by different absorbances, which were evaluated via a Cell Counting Kit-8 assay.

50% tissue culture infectious dose assay ($TCID_{50}$ Assay)

The different samples were diluted tenfold from 10^{-1} to 10^{-10}. The appropriate dilutions of the samples were then inoculated into the cells at 80% confluence, with eight replicates for each dilution. The $TCID_{50}$ results were obtained via the Reed-Muench method.

Viral titration assay

The monolayer of cells reached 80% confluence, and various concentrations of compounds (1, 2, 4, 6, 8 or 10 μM) were added for 2 h before virus inoculation. Two hours after PEDV infection, DMEM was added, and the mixture was incubated for 18 h before the lysate was collected for the $TCID_{50}$ assay.

Viral attachment and internalization assays

The cells were infected with different concentrations of compounds (5 or 10 μM) premixed with 0.1 MOI PEDV for 1 h at 4°C. After that, the culture medium was replaced with DMEM for 16 h. The results of the entry assay were analyzed via $TCID_{50}$ by collecting lysates.

To conduct an internalization assay of the virus, Vero cells were chilled at 4°C before being infected with 0.1 MOI PEDV and incubated at 4°C for 2 h. The Vero cells were subsequently exposed to DMEM supplemented with different concentrations of the test compounds (5 or 10 μM) to facilitate internalization. After 2 h, the

medium was replaced by DMEM. Lysates were collected to perform the $TCID_{50}$ assay.

Replication assay

To verify that the compounds inhibited the PEDV replication phase, cells at 80% confluence were incubated with different compounds (5 or 10 μM) after preinfection with 0.1 MOI PEDV for 2 h. Finally, the supernatants were collected to determine the $TCID_{50}$ assay.

Release assay

The cells were infected with 0.1 MOI PEDV for 18 h and then incubated with the test compounds for different durations (15, 30 and 60 min). The titer of PEDV in the lysate was subsequently detected via a $TCID_{50}$ assay.

Synergistic effect evaluation

The synergistic effect was evaluated via the SynergyFinder website (https://synergyfinder.fimm.fi) (Ianevski et al. 2020). This website enables the calculation of dose–response maps and the generation of synergistic score maps. The ZIP model was used to calculate the synergistic score. The most synergistic regions (MSA scores) can also be found in the synergistic interaction graph through SynergyFinder. The interaction was considered to be antagonistic when the synergy score fell below −10. When the score was between −10 and 10, it suggested an additive effect; and above 10, the interaction was likely synergistic.

Statistical analysis

Statistical evaluations were conducted by one-way ANOVA *via* GraphPad Prism V.8.0 (GraphPad, San Diego, CA, USA). $P<0.05$ was considered to indicate a significant difference. Significance levels are denoted as follows: * for $p<0.05$, ** for $p<0.01$, and *** for $p<0.001$.

Abbreviations

CC_{50}	Concentration of cytotoxicity 50%
CPE	Cytopathic effect
DMSO	Dimethyl sulfoxide
MOI	Multiplicity of infection
μM	μmmol/L

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s44149-025-00157-y.

Supplementary Material 1: Fig. S1. Inhibitory effects of gemcitabine on different phases of PEDV proliferation. Vero cells were incubated with gemcitabine (5,10 μM) and PEDV (MOI = 0.1) as described in the Methods section. (A) The antiviral effects of gemcitabine were evaluated via indirect immunofluorescence. Microscope scale: 100 μm. The effects of gemcitabine on the attachment phase (B), internalization phase (C) and release phase (D) of PEDV proliferation were assessed via $TCID_{50}$ assays. The data were analyzed with GraphPad Prism 8.0. The data are expressed as the means and standard deviations from three independent experiments and were analyzed via one-way ANOVA. ns, $p > 0.01$.

Supplementary Material 2: Fig. S2. The anti-PEDV activity of natural products. Vero cells were incubated with natural products (20 μM) and infected with PEDV (MOI = 0.1) for 18 h as described in the Methods section. The group treated with DMSO was used as a negative control. The infection rate of PEDV was subsequently determined via an immunofluorescence assay (A) and a $TCID_{50}$ assay (B). The data are expressed as the means and standard deviations from three independent experiments and were analyzed *via* one-way ANOVA. ns, $p>0.05$; **, $0.001 \leq p < 0.01$; ***, $p < 0.001$.

Supplementary Material 3: Fig. S3. The antiviral effects of baicalein on different phases of PEDV proliferation. Vero cells were incubated with baicalein (5,10 μM) and PEDV (MOI = 0.1) as described in the Methods section. (A) The antiviral effects of baicalein were evaluated by IFA. The microscope scale was 100 μm. The viral N protein is displayed in green, and the nuclei are displayed in blue. The experiments were performed three times and from three random horizons in each experiment. The effects of baicalein on the attachment phase (B), replication phase (C) and release phase (D) of PEDV proliferation were assessed via $TCID_{50}$ assays. The data were analyzed with GraphPad Prism V. 8.0. The data are expressed as the means and standard deviations from three independent experiments and were analyzed via one-way ANOVA. ns, $p>0.05$; *, $0.01 \leq p < 0.05$.

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Authors' contributions

The authors' responsibilities were as follows: WDG designed the research; ZYG, ZYF, XFY, and ZYJ finished this study and performed the data processing; the paper was written by ZYG; Dehua Luo and Yuxiang Wang assisted in revising the paper; and the final manuscript was approved by all the authors. WDG was primarily responsible for the content presented in the paper. The corresponding author of this paper is Dengguo Wei.

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Data availability

All data, including generation and analysis data, can be found in the paper and supplementary information.
All data of this article will be provided upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors state that the work reported in this article will not be affected by their personal relationships because there are no known conflicts of interest.

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