Antiviral effect of copper chloride on feline calicivirus and Synergy with Ribavirin in *vitro*

dengliang li (lidengliang2019@163.com)
Jilin Agricultural University
Zhanding Cui
Jilin Agricultural University
Guohua Li
Shihezi University
Liangting Zhang
Hebei Agricultural University
Ying Zhang
Northeast Forestry University
Han Zhao
Jilin Agricultural University
Shuang Zhang
Jilin Agricultural University
Yanbing Guo
Animal Husbandry and Veterinary Science Research Institute of Jilin Province
Yanli Zhao
Jilin Agricultural University
Fanxing Men
Jilin Agricultural University
Shihui Zhao
Jilin Agricultural University
Jiang Shao
Jilin Agricultural University
Dongju Du
Jilin Agricultural University
Hailong Huang
Jilin Agricultural University
Kai Wang
Jilin Agricultural University
Guixue Hu
Jilin Agricultural University
Tiansong Li
Beihua University
Yongkun Zhao
Chinese Academy of Agricultural Sciences

Research article

Keywords: Feline calicivirus, copper chloride, antiviral effect, synergistic protective effect, antagonistic effect

Posted Date: January 8th, 2020

DOI: https://doi.org/10.21203/rs.2.20385/v1

Version of Record: A version of this preprint was published at BMC Veterinary Research on July 6th, 2020. See the published version at https://doi.org/10.1186/s12917-020-02441-0.

Abstract

Background: Feline calicivirus (FCV) is a common pathogen causing widely prevalent upper respiratory disease for kitten and felines in recent years. Due to the substantial genetic variability of the viral genes, existing vaccines cannot provide complete protection. Therefore, researches on FCV antiviral drugs have received much attention.

Results: In this study, we found that copper chloride had dose-dependent antiviral effects against FCV in F81 cells. We also found that the combination of copper chloride and ribavirin had a synergistic effect against FCV in the F81 cells. In contrast, the combination of and horse anti-FCV immunoglobulin $F(ab')_2$ showed an antagonistic effect, likely because the copper chloride has an effect on the $F(ab')_2$ immunoglobulin; however, further research is needed to clarify this supposition.

Conclusions: In summary, we found that copper chloride had low cytotoxicity and significant antiviral effects against FCV in F81 cells, providing a new drug candidate for the prevention and treatment of FCV infection.

Background

Feline calicivirus (FCV) is a small, nonenveloped, single positive-strand RNA virus that belongs to the genus Vesivirus of the family Caliciviridae [1]. Most felines (e.g., cats, tigers, and cheetahs) are susceptible, and infection in dogs has been reported in recent years [2, 3]. Kittens aged 1 to 12 weeks are mainly infected, with a mortality rate of 67% [4, 5]. Infected animals present with oral ulcers, chronic stomatitis, rhinitis, conjunctivitis, and pneumonia [6–9]. The primary prevention at present remains vaccination. However, due to the high evolutionary rate of the FCV capsid protein, with as many as 1.3×10^{-2} to 2.6×10^{-2} substitutions per nucleotide per year [10], traditional vaccination cannot completely protect the animals from re-infection of mutant or wild-type strains, even if the FCV was considered to have only one serotype [11]. Therefore, it is necessary to develop a safe and effective antiviral drug as a monotherapy or as part of a combination treatment.

In previous studies, various compounds had been found to have anti-FCV effects, such as minomorpho oligophosphate (PMO), lithium chloride, and germacrone [12–14]. The combination of mefloquine and recombinant cat interferon-ω had a synergetic effect against FCV [15]. Copper is an indispensable element used in the production of livestock and poultry. Basic copper chloride is an essential additive in feed. Researchers previously found that copper and copper compounds had antiviral effects against dengue virus, influenza virus, and human immunodeficiency virus (HIV) in vitro [16–18]. The copper(II) chloride complex has also been used for anticancer effects in vitro in human cell lines of cervical cancer, colon cancer, ovarian cancer, and melanoma [19]. However, there have been no reports on the antiviral effect of copper chloride against FCV.

In this study, we found that copper chloride induces low cytotoxicity in F81 cells. We evaluated the antiviral effects of copper chloride in vitro in a dose-dependent manner by measuring the $TCID_{50}$ and using RT-qPCR to analyse the effects against FCV. Additionally, the combination of copper chloride and ribavirin showed synergistic antiviral effects against FCV.

Results

Cytotoxicity test of copper chloride on F81 cells

The results of the cytotoxicity assay showed that the relative cell viability was above 85% after treatment with copper chloride at a concentration of 20~80 μM for 24 h or 72 h. When the concentration of the copper chloride was not higher than that of 200 μM (below CC_{50}), the relative cell viability of the F81 cells was higher than that of 50% after 24 h or 72 h

of treatment, indicating that copper chloride can be regarded as non-toxic in this concentration range. Therefore, a concentration of 200 μM copper chloride was used as the maximum concentration for the antiviral experiments.

Fig. 1 Cytotoxicity assay of copper chloride to F81 cells. A CCK-8 assay was used to measured cytotoxicity to F81 cells exposed to copper chloride at concentrations of 400, 200, 100, 80, 60, 40, and 20 μM during incubation at 37 °C and 5% CO_2 for 24 h or 72 h. The relative activity of 0.4% DMSO-treated F81 cells was considered to be 100%, and the cytotoxicity was shown as the percentage of cell activity with respect to the DMSO mimetic treatment. Each value represents three independent replicate experiments.

Antiviral effect of copper chloride at different doses

To evaluate the antiviral effects of different doses of copper chloride against FCV, we examined the virus titre and RNA levels of FCV in the F81 cells treated with different doses of copper chloride. The results showed that the virus titre was significantly lower than that of the mock group after the cells were exposed to 60, 80, 100, and 200 μM concentrations of copper chloride ($p < 0.001$) (Fig. 2A). The relative RNA levels of the FCV were significantly reduced compared to the mock group when the copper chloride concentration was 80 or 100 μM ($p < 0.005$), and the decrease was very significant at 200 μM ($p < 0.001$) (Fig. 2B). In addition, all the results indicated that the antiviral effect of copper chloride on the F81 cells was dose-dependent. The IC_{50} of the copper chloride to FCV was determined to be 5.1 μM (Fig. 2C).

Fig. 2 The antiviral effect of different doses of copper chloride (20-200 μM) on the 100 $TCID_{50}$ FCV-infected F81 cells. After incubation for 28 h at 37 ℃ and 5% CO_2, the virus titre (A) and the relative RNA levels (B) of the FCV were detected. (C) The IC_{50} of the copper chloride to the FCV was determined; * $p < 0.0332$; ** $p < 0.0021$; *** $p < 0.0002$; and **** $p < 0.0001$.

Indirect immunofluorescence assay

To further evaluate whether the antiviral effect of copper chloride against FCV was dose-dependent, we performed an indirect immunofluorescence assay (IFA). The results showed that intense green fluorescence signal was observed in the 0 μM group, and only weak fluorescence signals were observed at the concentrations of 60, 40, and 20 μM, indicating a dose-dependent relationship after greyscale scanning (Fig. 3B). There was almost no fluorescence signal at the concentrations of 80, 100, and 200 μM or for the control group compared to the mock group (Fig. 3A). In addition, the F81 cells treated with 200 μM copper chloride showed slight rounding and polymerization. This finding also validates the range of non-toxic concentrations of copper chloride previously described.

Fig. 3 IFA verified the antiviral effect of copper chloride. (A) F81 cells were infected with different concentrations of copper chloride (20-200 μM) and FCV (100 $TCID_{50}$), and a mock treatment group containing 0.4% DMSO and a negative control group uninfected with FCV were used as controls. After incubation for 1 h at 37°C and 5% CO_2, IFA of the F81 cells was performed. (B) The antiviral effect of the copper chloride was evaluated by scanning the fluorescence signal; **** $p < 0.0001$.

Antiviral effect of copper chloride for different treatment times

To evaluate the antiviral effect of copper chloride to FCV at different treatment times, we examined the virus titer and RNA levels of FCV in F81 cells at different action times. The results showed that the virus titre and the relative RNA level of the FCV were significantly lower in the copper chloride treatment group after -1, 0, and 1 h of FCV infection compared with that of the mock group ($p < 0.001$). After 16 h of virus infection, there was no significant difference between the virus titre or relative RNA level of the FCV and the mock group, indicating that the antiviral effect of copper chloride (80 μM) was not significant after 8 h of FCV infection (Fig. 4A and B). The RT-qPCR data also obtained the similar results.

Fig. 4 The antiviral effect of copper chloride on FCV at different times. The F81 cells were infected with 100 $TCID_{50}$ FCV and then treated with 80 μM copper chloride for -1, 0, 1, 2, 4, 8, and 16 h. After incubation for 24 h at 37 °C and 5% CO_2, the virus titre (A) and relative RNA levels (B) of the FCV were detected. NS, $p > 0.1234$; ** $p < 0.0021$; *** $p < 0.0002$; and **** $p < 0.0001$.

Antiviral effect of copper chloride on different strains

For other strains of FCV, the antiviral effects of copper chloride were validated. As previously described, we determined the viral titre and relative viral RNA levels of the different strains after copper chloride treatment. The results showed that 80 μM copper chloride used to treat the other strains of FCV (CH-JL1, CH-JL3, CH-JL4, and CH-SH) isolated in ourlaboratory before[20] significantly reduced the viral titre and relative viral RNA levels of the infected F81 cells (Fig. 5A and B). These results indicate that copper chloride has a strong antiviral effect against strains of FCV with differing isolates.

Fig. 5 The antiviral effect of copper chloride for different FCV strains. The F81 cells were infected with different FCV strains at 100 $TCID_{50}$ and treated with 80 μM copper chloride. After incubation for 28 h at 37 °C and 5% CO_2, the virus titre (A) and the relative RNA levels (B) of the FCV were detected; * $p < 0.1234$ and ** $p < 0.0021$.

Synergistic effect of ribavirin and the antagonistic effect of $F(ab')_2$

The checkerboard method was used to mix different compounds, and the antiviral effect of the compound combination was evaluated using SynergyFinder. The results showed that the combination of copper chloride and ribavirin had a synergistic effect on the protection of FCV-infected F81 cells within a specific concentration range (Fig. 6A). The ZIP model was used to calculate the average score for the synergy of the two compounds, and it was found to be 9.687 (Fig. 6B). Conversely, the combined use of copper chloride and $F(ab')_2$ showed antagonism, and the average score of the two compounds calculated by the ZIP model was -20.798 (Fig. 6C and D).

Fig. 6 The antiviral effect of the compound combination. (A and C) The compounds were diluted to the indicated concentrations and used in combination to treat FCV infection. The results of the RT-qPCR were statistically analyzed by the methods described above, and the effects of the drug combination were evaluated using SynergyFinder. (B and D) Different concentrations of compound interaction scores were calculated using the ZIP model.

Discussion

In previous studies, copper or copper ions had an antiviral effect on herpes simplex virus (HSV) [21]. However, they were mainly used to inactive virions since copper ions can destroy the activity of certain hydroxyl radical (OH)-containing proteins [22, 23]. However, when the concentration of the copper ions was too high, its toxicity to the organisms was fatal because copper chloride can inactivate specific proteins and induce a large number of reactive oxygen species in cells to induce apoptosis [24]. In our study, we found that low concentrations of copper chloride induced low cytotoxicity in the F81 cells. The F81 cells treated with copper chloride concentrations of less than 200 μM for 24 h and 72 h showed cell activity greater than 50%, which was similar to previously reported soluble copper chloride cytotoxicity results [25]. Previously, copper chloride dihydrate-treated DENV exhibited excellent antiviral activity but the antiviral effect of copper chloride on FCV had not been reported [26]. In this study, we found that copper chloride had an antiviral effect against FCV with an IC50 of 5.1 μM. The IFA results showed that the inhibition of FCV by copper chloride was dose-dependent and significantly reduced the cytopathic effect (CPE) caused by FCV in the F81 cells. After treatment with different concentrations of copper chloride, the viral titre and RNA expression levels of FCV were significantly different from those of the control.

Then, we demonstrated the inhibitory effect of copper chloride on the viral titre and RNA levels of FCV at different times. FCV was significantly inhibited when copper chloride was added 8 h before FCV was used to infect the cells, but copper chloride did not significantly inhibit FCV after 16 h. Because our findings differed from those of previous studies using fexaramine, LiCl, ginger extracts,or CSX [13, 14, 27, 28], we speculated that copper chloride can inhibit the replication of FCV in cells and does not affect the adsorption or entry of the virus into the cells, but its mechanism of inhibiting replication needs further verification. We found that cupric chloride also inhibited other strains of FCV replication in the F81 cells, which is further evidence of the antiviral activity of copper chloride against FCV. In a later experiment, we found that the broad-spectrum antiviral drugs ribavirin and copper chloride have synergistic effects in inhibiting FCV. The most synergistic area scored 15.5, with an average score of 9.7. However, copper chloride and an immunoglobulin $F(ab')_2$ showed a robust antagonistic effect. The cupric chloride may affect the biological activity of $F(ab')_2$ [29], resulting in antagonistic effects of the two drugs in cells infected with FCV, but more proof is needed.

Conclusions

In summary, we verified that copper chloride induced low cytotoxicity in the F81 cells and had an antiviral effect on FCV in vitro. Later, we found that copper chloride played a role in the replication stages of FCV-infected cells, and it had a synergistic effect with ribavirin, but the drug resistance should be determined in a future study. These studies further confirmed that copper chloride could be used as one of candidate drugs for developing anti-FCV agents.

Methods

Viruses, cells and compounds

The FCV CH-SH strains and F81 cells were provided by the Institute of Military Veterinary Medicine, Academy of Military Medical Science. FCV CH-JL1/CH-JL2/CH-JL3/CH-JL4 were isolated and stored in our laboratory [20]. The horse anti-FCV immunoglobulin $F(ab')_2$ was produced and stored in our laboratory [30]. The cupric chloride dihydrate (Aladdin, China) product number was C111685. The ribavirin (Aladdin, China) product number was R101754.

Cytotoxicity assay for copper chloride

Diluted copper chloride (100 μL per well) was added to the F81 cells at 100% confluence in a 96-well plate, and MEM containing 0.4% DMSO was used as the control. The cells were incubated at 37 ℃ and 5% CO_2 for 24 h or 72 h. After washing the cells twice with PBS solution, 180 μL of FBS-free MEM and 20 μl of CCK-8 (CCK8; Dojindo, Japan) were added to each well. After incubation at 37 ℃ for 1-2 h, the optical density (OD) was read at 450 nm using a Cmax Plus microplate reader (Molecular Devices, USA). The cell viability was calculated by the formula [OD_{450} (compound) - OD_{450} (blank)] / [OD_{450} (control) - OD_{450} (blank)] × 100%. The copper chloride concentrations that were 50% or less than the cytostatic concentration (CC_{50}) were defined as non-toxic [31].

Virus titre and genome detection

The virus solution to be detected was diluted in a 10-fold gradient. Each concentration was added to each column at 100 μL per well, followed by the addition of 100 μL of 2% MEM. The control was established in virus-free medium and cultured at 37 °C and 5% CO_2. The virus $TCID_{50}$ was calculated using the Reed and Muench formula. Relative RT-qPCR was used to evaluate FCV gene expression. Briefly, first, the RNA of the virus was extracted by the Simply P total RNA extraction kit (Bioflux, China), and then, the RNA was reverse transcribed into complementary DNA (cDNA) (Thermo, USA), followed by RT-qPCR using TB Green Premix Ex Taq II (Tli RNaseH Plus) (TaKaRa，China). The upstream and

downstream sequences for FCV were 5'-GCAGGTTGGGATAAACATGGA-3' and 5'-CACGAGGCGATTGAGTTGAG-3', and for GAPDH, they were 5’-TGGAAAGCCCATCACCATC-3’ and 5’-ACTCCACAACATACTCAGCACCA-3’.

Antiviral effect of copper chloride on FCV

F81 cells at 100% confluence were infected with 100 $TCID_{50}$ of FCV CH-JL2, while different concentrations of copper chloride (200~20 μM) were added to the medium. The MEM containing 0.4% DMSO was used as a mock treatment group. After incubation for 28 h at 37 °C and 5% CO_2, the $TCID_{50}$ levels of virus and the RT-qPCR results were determined to assess the antiviral effect against FCV. The half-maximal inhibitory concentration (IC_{50}) of the copper chloride to FCV was also determined, and the results were plotted using GraphPad Prism 8.

Indirect immunofluorescence assay (IFA)

To more intuitively observe the antiviral effect of copper chloride on FCV, we performed an IFA [30]. As described above, a monolayer of F81 cells infected with 100 $TCID_{50}$ of FCV was exposed to different concentrations of copper chloride and then fixed in 80% cold acetone for 30 min. Next, the cells were washed 5 times with PBS containing 0.05% Tween-20 (PBST). Then, primary antibody (VMRD, USA) diluted 300-fold with 1% BSA was added and incubated for 1 h at 37 °C. The supernatant was discarded, and the cells washed 5 times with PBST, and a 200-fold diluted FITC-labelled rabbit anti-cat secondary antibody (Bioss Antibody, China) was added in the dark and incubated at 37 °C for 1 h. The fluorescence was observed under an inverted Leica DMi8 fluorescence microscope (Leica, Germany), and the fluorescence results were analyzed by greyscale scanning.

Effect of different treatment times of copper chloride on FCV

To determine whether the antiviral effect of copper chloride in F81 cells was time-dependent, a final concentration of 80 μM copper chloride solution was administered -1 h, 0 h, 2 h, 4 h, 6 h, 8 h, and 16 h after FCV infection. After incubation for 24 h at 37 °C and 5% CO_2, the $TCID_{50}$ level was determined and the RT-qPCR results were assessed to determine the amount of virus.

Antiviral effect of copper chloride on different strains

To determine whether copper chloride also has antiviral effects against other FCV strains, we also diluted different strains of FCV (CH-JL1, CH-JL3, CH-JL4, and CH-SH) to 100 $TCID_{50}$ and infected the F81 cells, which were subsequently treated them with a final concentration of 80 μM copper chloride solution. After incubating the cells with virus for 28 h at 37 °C and 5% CO_2, the $TCID_{50}$ level was determined and the RT-qPCR results were assessed to determine the amount of virus.

Combinations of copper chloride and ribavirin or $F(ab')_2$

To evaluate the combined action of the compounds, we diluted the different drugs by the checkerboard method and mixed the two drugs thoroughly (copper chloride and ribavirin or copper chloride and $F(ab')_2$). The results from the RT-qPCR experiments were statistically analyzed in a manner described previously, and the effects of the drug combination were evaluated using SynergyFinder [32]. The zero-interaction-efficiency (ZIP) model [26]was used to calculate the mutual scores of the different concentrations of the drug. For each treatment, at least three triplicate data sets from independent experiments were analyzed. The results are expressed as the mean and mean standard error (SEM).

Statistical methods

All experiments were performed three times independently in triplicate. The data were expressed as the mean ± standard deviation (SD). The significance of the differences between the groups was determined by paired t-test and one-way/two-way analysis of variance.

Abbreviations

PMO
minomorpho oligophosphate; $TCID_{50}$:50% tissue culture infective dose; RT-qPCR:Real time quantitative polymerase chain reaction; CC_{50}:50% cytostatic concentration;PBS:phosphate-buffered saline; IC_{50}:the half-maximal inhibitory concentration; IFA:Indirect immunofluorescence assay; SEM:Standard error of the mean; FITC:fluoresceine isothiocyanate; ZIP:the zero-interaction-efficiency

Declarations

Acknowledgement

The authors would like to thank Xiaoxueying Chen, Yue Teng, Yuxing Tan(Jilin Agricultural University) for invaluable technical assistance.

Author Contributions: Dengliang Li, Zhanding Cui and Guixue Hu conceived and designed the experiments. Dengliang Li and Zhanding Cui performed the experiments. Dengliang Li and Zhanding Cui analyzed the data. Guohua Li, Liangting Zhang, Ying Zhang, Han Zhao, Shuang Zhang, Yanbing Guo, Yanli Zhao, Fanxing Men, Shihui Zhao, Jiang Shao, Dongju Du, Hailong Huang, Kai Wang contributed reagents/materials/analysis tools. Dengliang Li and Zhanding Cui wrote the paper. Guixue Hu, Tiansong Li and Yongkun Zhao requested financial support. All authors read and approved the manuscript.

Funding

The present work was supported by the National Key R&D Program for the 13th Five-Year Plan, the Ministry of Science and Technology of China and the National Key R&D Program (2016YFD0501002).

Availability of data and materials

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

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]College of Animal Science and Technology, Jilin Agricultural University, Changchun, 130118, China. [2]Key Laboratory of Jilin Province for Zoonosis Prevention and Control, Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Changchun 130122, Jilin, China. [3]CCollege of Animal Science and Technology, Shihezi University, Shihezi 832003, China. [4]College of Continuing Education, Hebei Agricultural University, Baoding, 071001, China. [5]College of Wildlife and Protected Area Northeast Forestry University, Harbin, 150040,China.[6]Animal Husbandry and Veterinary Science Research Institute of Jilin Province, Changchun 130062, China. [7]College of Science, Beihua University, Jilin, 132013, China.

References

1.
Clarke IN, Lambden PR. The molecular biology of caliciviruses. J GEN VIROL. 1997;78(Pt 2):(2):291–301.
2.
Di Martino B, Di Rocco C, Ceci C, Marsilio F. Characterization of a strain of feline calicivirus isolated from a dog faecal sample. VET MICROBIOL. 2009;139(1–2):52–7.
3.
Martella V, Pratelli A, Gentile M, Buonavoglia D, Decaro N, Fiorente P, Buonavoglia C. Analysis of the capsid protein gene of a feline-like calicivirus isolated from a dog. VET MICROBIOL. 2002;85(4):315–22.
4.
Battilani M, Vaccari F, Carelle MS, Morandi F, Benazzi C, Kipar A, Dondi F, Scagliarini A. Virulent feline calicivirus disease in a shelter in Italy: a case description. RES VET SCI. 2013;95(1):283–90.
5.
Dedication. In: Consultations in Feline Internal Medicine (Fifth Edition). Edited by August JR. Saint Louis: W.B. Saunders; 2006: v.
6.
Sykes JE. Pediatric feline upper respiratory disease. Vet Clin North Am Small Anim Pract. 2014;44(2):331–42.
7.
Hurley KF, Sykes JE. Update on feline calicivirus: new trends. Vet Clin North Am Small Anim Pract. 2003;33(4):759–72.
8.
Cohn LA. Feline respiratory disease complex. Vet Clin North Am Small Anim Pract. 2011;41(6):1273–89.
9.
Willi B, Spiri AM, Meli ML, Samman A, Hoffmann K, Sydler T, Cattori V, Graf F, Diserens KA, Padrutt I, et al. Molecular characterization and virus neutralization patterns of severe, non-epizootic forms of feline calicivirus infections resembling virulent systemic disease in cats in Switzerland and in Liechtenstein. VET MICROBIOL. 2016;182:202–12.
10.
Coyne KP, Gaskell RM, Dawson S, Porter CJ, Radford AD. Evolutionary mechanisms of persistence and diversification of a calicivirus within endemically infected natural host populations. J VIROL. 2007;81(4):1961–71.
11.
Radford AD, Coyne KP, Dawson S, Porter CJ, Gaskell RM. Feline calicivirus. VET RES. 2007;38(2):319–35.
12.
Smith AW, Iversen PL, O'Hanley PD, Skilling DE, Christensen JR, Weaver SS, Longley K, Stone MA, Poet SE, Matson DO. Virus-specific antiviral treatment for controlling severe and fatal outbreaks of feline calicivirus infection. AM J VET RES. 2008;69(1):23–32.
13.
Wu H, Zhang X, Liu C, Liu D, Liu J, Tian J, Qu L. Antiviral effect of lithium chloride on feline calicivirus in vitro. ARCH VIROL. 2015;160(12):2935–43.

14.
Wu H, Liu Y, Zu S, Sun X, Liu C, Liu D, Zhang X, Tian J, Qu L. In vitro antiviral effect of germacrone on feline calicivirus. ARCH VIROL. 2016;161(6):1559–67.
15.
McDonagh P, Sheehy PA, Fawcett A, Norris JM. Antiviral effect of mefloquine on feline calicivirus in vitro. VET MICROBIOL. 2015;176(3–4):370–7.
16.
Horie M, Ogawa H, Yoshida Y, Yamada K, Hara A, Ozawa K, Matsuda S, Mizota C, Tani M, Yamamoto Y, et al. Inactivation and morphological changes of avian influenza virus by copper ions. ARCH VIROL. 2008;153(8):1467–72.
17.
Shahabadi N, Abbasi AR, Moshtkob A, Shiri F. DNA-binding studies of a new Cu(II) complex containing reverse transcriptase inhibitor and anti-HIV drug zalcitabine. Journal of Coordination ChemistryJournal of Coordination Chemistry. 2019;72(12):1957–72.
18.
Sucipto TH, Churrotin S, Setyawati H, Kotaki T, Martak F, Soegijanto S. ANTIVIRAL ACTIVITY OF COPPER(II) CHLORIDE DIHYDRATE AGAINST DENGUE VIRUS TYPE-2 IN VERO CELL. Indonesian Journal of Tropical Infectious Disease. 2017;6(4):84–7.
19.
Jopp M, Becker J, Becker S, Miska A, Gandin V, Marzano C, Schindler S. Anticancer activity of a series of copper(II) complexes with tripodal ligands. EUR J MED CHEM. 2017;132:274–81.
20.
Zhao Y, Chen X, Ying Y, Wang K, Dong H, Gao C, Yang S, Hu G. Isolation and phylogenetic analysis of three feline calicivirus strains from domestic cats in Jilin Province, China. ARCH VIROL 2017, 162(9).
21.
Sagripanti JL, Routson LB, Bonifacino AC, Lytle CD. Mechanism of copper-mediated inactivation of herpes simplex virus. Antimicrob Agents Chemother. 1997;41(4):812–7.
22.
Betanzos-Cabrera G, Rez FJR, Oz JLM, Barrè´n BL, Maldonado R. Inactivation of HSV-2 by ascorbate-Cu(II) and its protecting evaluation in CF-1 mice against encephalitis. J VIROL METHODS. 2004;120(2):161–5.
23.
Sagripanti JL, Routson LB, Bonifacino AC, Lytle CD. Mechanism of copper-mediated inactivation of herpes simplex virus. 1997, 41(4):812.
24.
Wen-Jie G, Si-Si Y, Ning C, Juan H, Jing G, Qiu-Yun C. ROS-mediated autophagy was involved in cancer cell death induced by novel copper(II) complex. 2010, 62(5):577–582.
25.
Strauch BM, Niemand RK, Winkelbeiner NL, Hartwig A. Comparison between micro- and nanosized copper oxide and water soluble copper chloride: interrelationship between intracellular copper concentrations, oxidative stress and DNA damage response in human lung cells. PART FIBRE TOXICOL. 2017;14(1):17–28.
26.
Yadav B, Wennerberg K, Aittokallio T, Tang J. Searching for Drug Synergy in Complex Dose-Response Landscapes Using an Interaction Potency Model. Computational Structural Biotechnology Journal. 2015;13(C):504–13.
27.
Kim Y, Chang K. Fexaramine as an entry blocker for feline caliciviruses. ANTIVIR RES. 2018;152:76–83.
28.

Aboubakr HA, Nauertz A, Luong NT, Agrawal S, El-Sohaimy SAA, Youssef MM, Goyal SM. In Vitro Antiviral Activity of Clove and Ginger Aqueous Extracts against Feline Calicivirus, a Surrogate for Human Norovirus. J FOOD PROTECT. 2016;79(6):1001–12.
29.
Smith MA, Easton M, Everett P, Lewis G, Payne M, Riveros-Moreno V, Allen G. Specific cleavage of immunoglobulin G by copper ions. International Journal of Peptide Protein Research. 2010;48(1):48–55.
30.
Cui Z, Li D, Yi S, Guo Y, Dong G, Niu J, Zhao H, Zhang Y, Zhang S, Cao L, et al: Equine immunoglobulin F(ab') 2 fragments protect cats against feline calicivirus infection. INT IMMUNOPHARMACOL 2019, 75.
31.
Jasenosky LD, Cadena C, Mire CE, Borisevich V, Haridas V, Ranjbar S, Nambu A, Bavari S, Soloveva V, Sadukhan S, et al: The FDA-Approved Oral Drug Nitazoxanide Amplifies Host Antiviral Responses and Inhibits Ebola Virus. iScience 2019, 19:1279–1290.
32.
Ianevski A, He L, Aittokallio T, Tang J. SynergyFinder: a web application for analyzing drug combination dose-response matrix data. Bioinformatics. 2017;33(15):2413–5.

Highlights

Copper chloride has antiviral activity against FCV.

Copper chloride and ribavirin have synergistic anti-FCV effects.

Copper chloride and horse immunoglobulin $F(ab')_2$ have antagonistic effects against FCV.

Figures

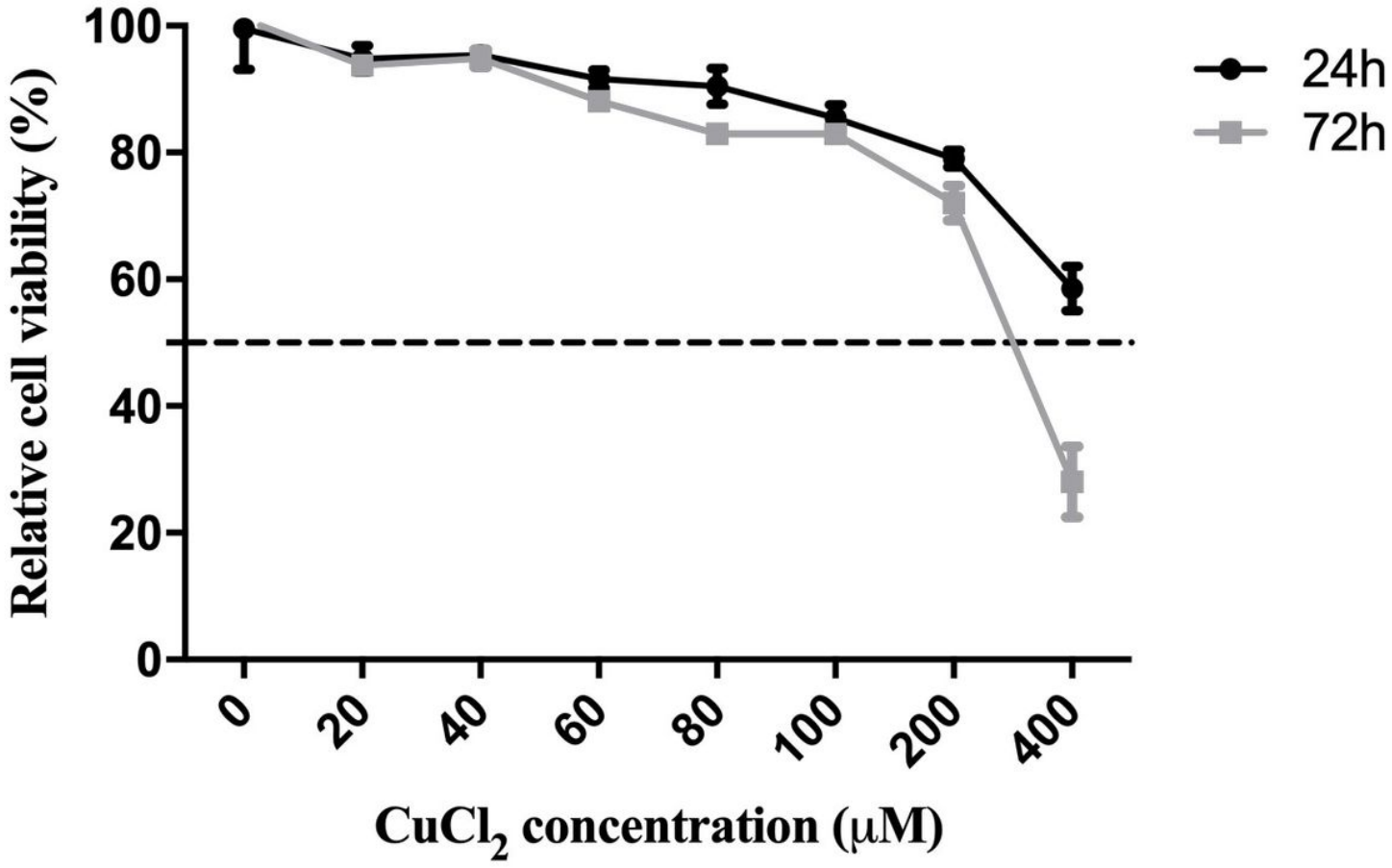

Figure 1

Cytotoxicity assay of copper chloride to F81 cells. A CCK-8 assay was used to measured cytotoxicity to F81 cells exposed to copper chloride at concentrations of 400, 200, 100, 80, 60, 40, and 20 μM during incubation at 37 °C and 5% CO2 for 24 h or 72 h. The relative activity of 0.4% DMSO-treated F81 cells was considered to be 100%, and the cytotoxicity was shown as the percentage of cell activity with respect to the DMSO mimetic treatment. Each value represents three independent replicate experiments.

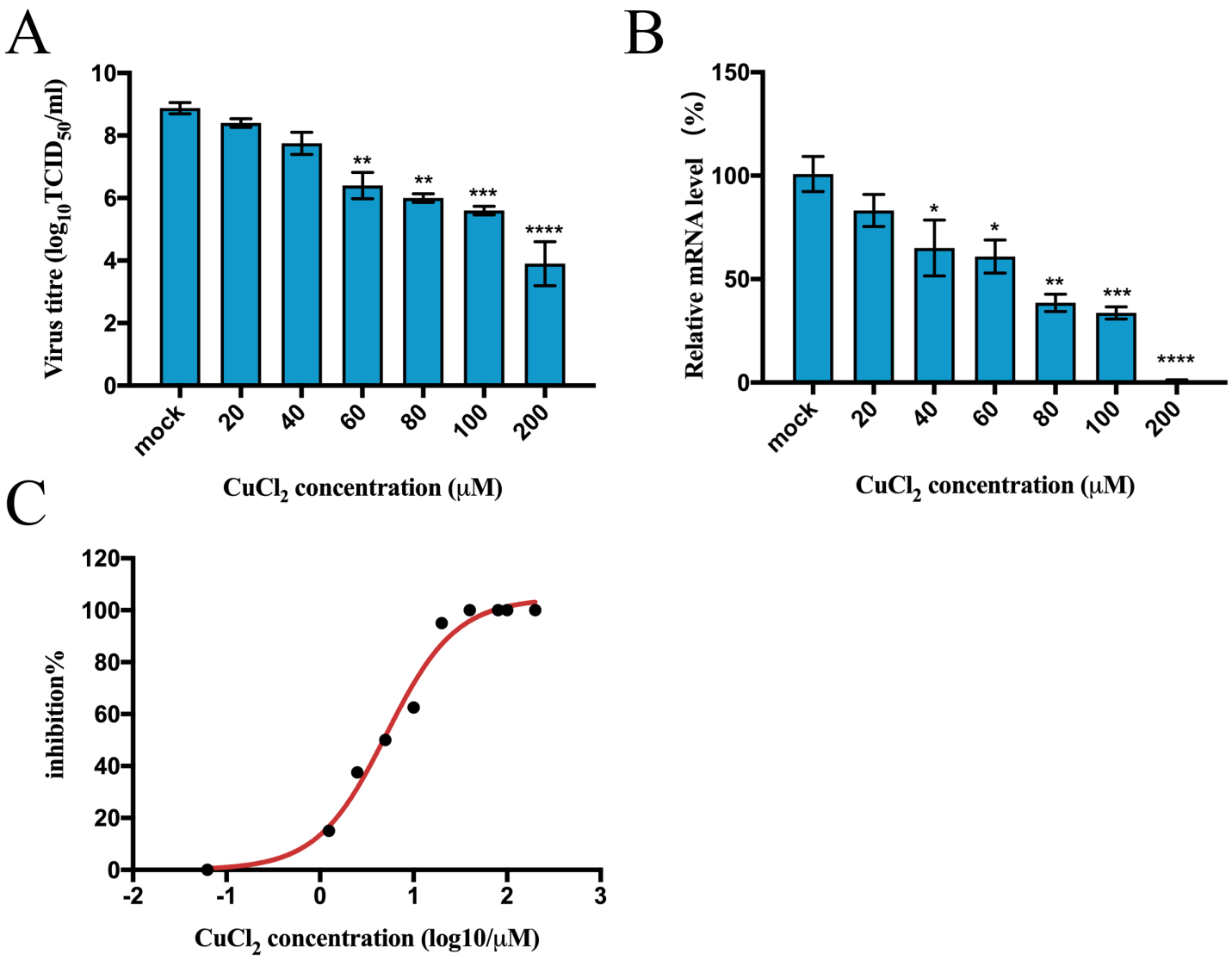

Figure 2

The antiviral effect of different doses of copper chloride (20-200 μM) on the 100 TCID50 FCV-infected F81 cells. After incubation for 28 h at 37 °C and 5% CO2, the virus titre (A) and the relative RNA levels (B) of the FCV were detected. (C) The IC50 of the copper chloride to the FCV was determined; * p <0.0332; ** p <0.0021; *** p <0.0002; and **** p <0.0001.

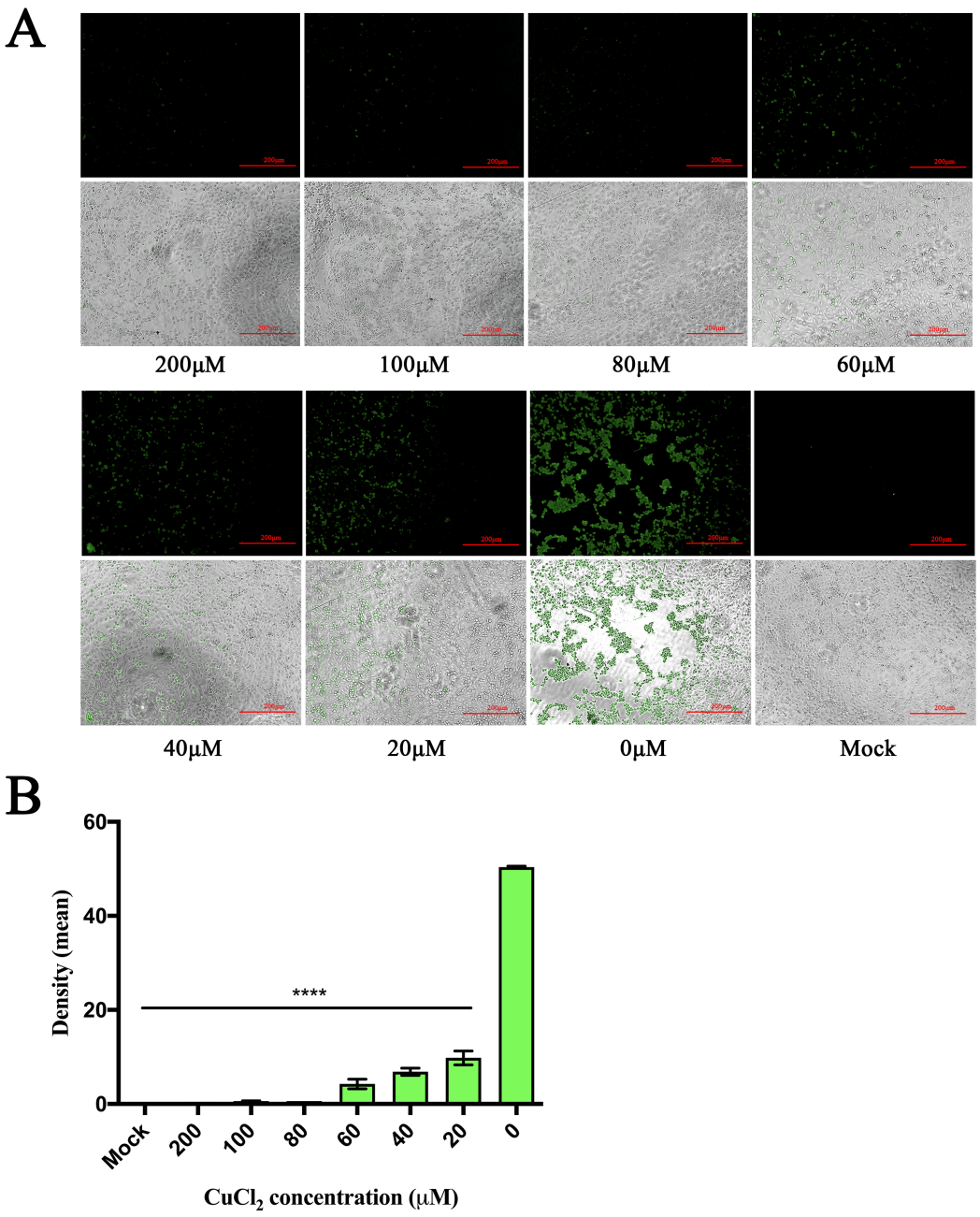

Figure 3

IFA verified the antiviral effect of copper chloride. (A) F81 cells were infected with different concentrations of copper chloride (20-200 μM) and FCV (100 TCID50), and a mock treatment group containing 0.4% DMSO and a negative control group uninfected with FCV were used as controls. After incubation for 1 h at 37°C and 5% CO2, IFA of the F81 cells was performed. (B) The antiviral effect of the copper chloride was evaluated by scanning the fluorescence signal; **** p <0.0001.

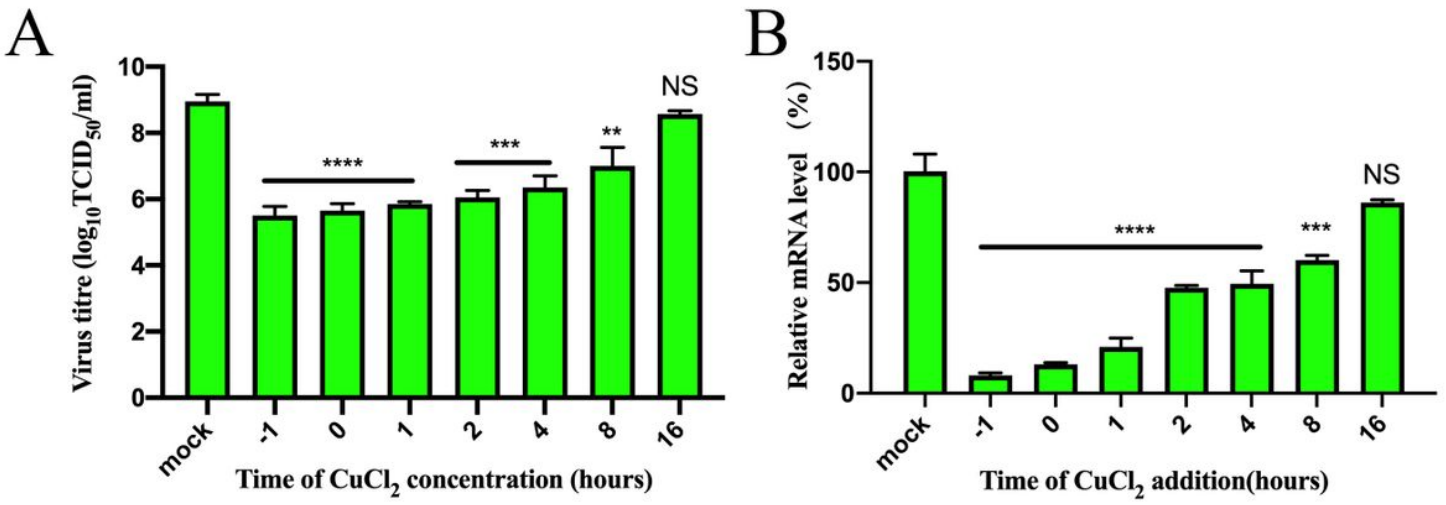

Figure 4

The antiviral effect of copper chloride on FCV at different times. The F81 cells were infected with 100 TCID50 FCV and then treated with 80 μM copper chloride for -1, 0, 1, 2, 4, 8, and 16 h. After incubation for 24 h at 37 °C and 5% CO2, the virus titre (A) and relative RNA levels (B) of the FCV were detected. NS, p > 0.1234; ** p <0.0021; *** p <0.0002; and **** p <0.0001.

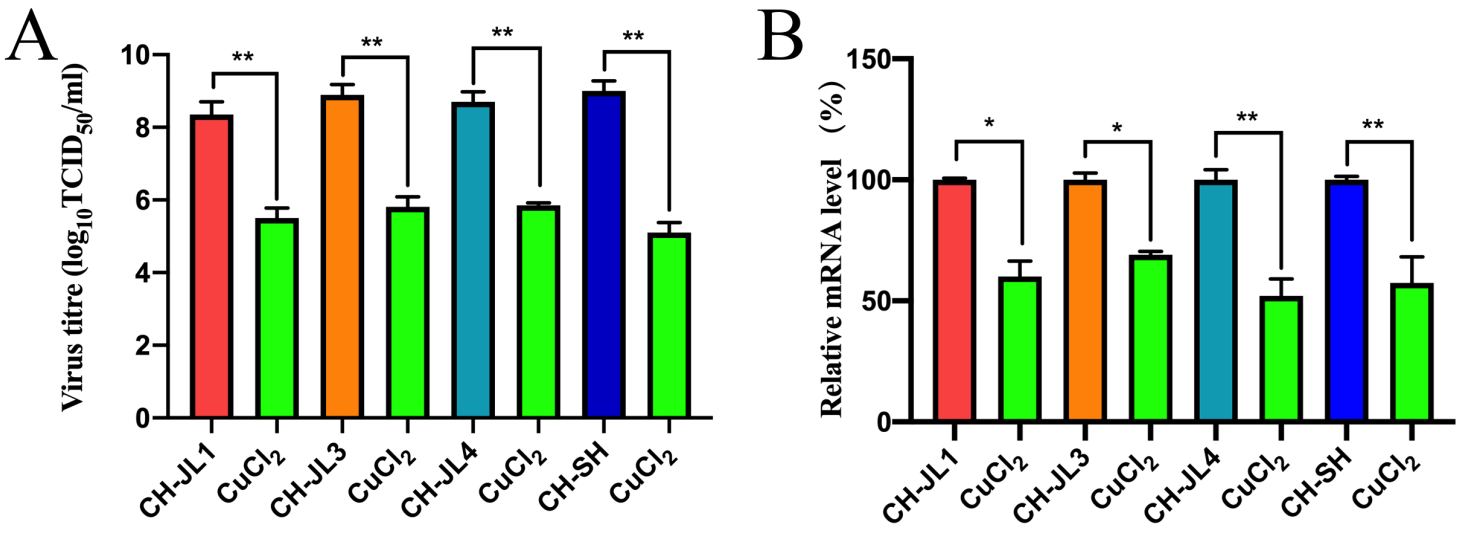

Figure 5

The antiviral effect of copper chloride for different FCV strains. The F81 cells were infected with different FCV strains at 100 TCID50 and treated with 80 μM copper chloride. After incubation for 28 h at 37 °C and 5% CO2, the virus titre (A) and the relative RNA levels (B) of the FCV were detected; * p < 0.1234 and ** p < 0.0021.

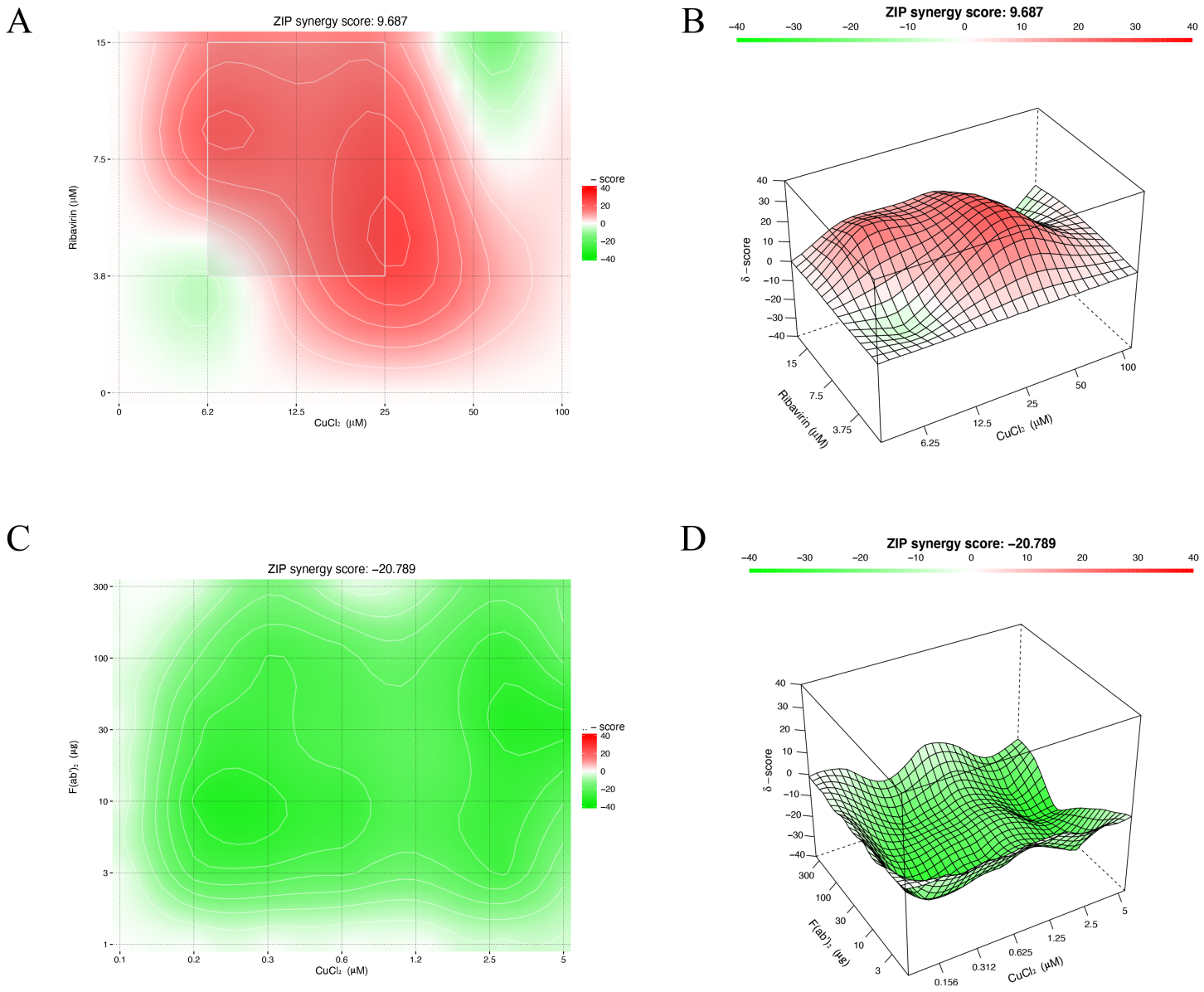

Figure 6

The antiviral effect of the compound combination. (A and C) The compounds were diluted to the indicated concentrations and used in combination to treat FCV infection. The results of the RT-qPCR were statistically analyzed by the methods described above, and the effects of the drug combination were evaluated using SynergyFinder. (B and D) Different concentrations of compound interaction scores were calculated using the ZIP model.