Antiviral effects of artemisinin and its derivatives

Hao Liu[1], Zhen Li[1,2], Yang Zhang[1,3], Lin Jia[1], Miaotian Cai[4], Rui Wang[1,2], Caiping Guo[1]

[1]Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China;
[2]Beijing Key Laboratory for HIV/AIDS Research, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China;
[3]Department of Dermatology, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China;
[4]Department of Respiratory and Critical Care Medicine, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China.

To the Editor: Artemisinin is a sesquiterpene lactone compound with a peroxide bridge structure, which is extracted and isolated from the medicinal plant, *Artemisia annua L*. Existing studies have revealed a broad spectrum of potential applications of artemisinin compounds in antitumor, immune regulation, osteoporosis prevention, antibacterial and antiviral roles over the years, making it effective against a range of other diseases, which broadened the clinical applications of artemisinin. Herein, we update the current knowledge of the antiviral activities of artemisinin and its derivatives against diverse viruses such as *Coronaviridae*, *Herpesviridae*, and *Flaviviridae*. Studies on the antiviral effect of artemisinin-type compounds can provide new ideas to fight the emerging viral disease for which no effective antiviral drugs are available.

Coronaviridae. In systematic classification, coronaviruses (CoVs) belong to the family of Coronaviridae, the order Nidovirales, and the genus Coronavirus. They are enveloped viruses with positive-sense, single-stranded ribonucleic acid (RNA) with genome sizes ranging from 26 kb to 32 kb. Like hydroxychloroquine (HCQ), artemisinin-based compounds can prevent the docking process of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein receptor-binding-domain to bind with the human receptor angiotensin-converting enzyme 2 by selectively interacting with the Lys353 and Lys31 binding hotspots via forming hydrogen bonds. But, artemisinin and derived compounds produced better Vina docking score (−7.1 kcal/mol for artelinic acid *vs.* −5.5 kcal/mol for HCQ) and much lower inhibition constant (Ki) than HCQ.[1] In a 41-person clinic trial, artemisinin combined with piperaquine (ART-PQP) was shown to be therapeutic in SARS-CoV-2-positive patients. The administration of ART-PQP reduced viral titers to undetectable levels by day 21, whereas patients in the control group cleared the virus on day 36.[2] However, the study did not rule out the interference of other antivirals; many patients also received multiple other treatments, including interferon (IFN)-α-1b, ribavirin, oseltamivir, lopinavir, and carrimycin.

Herpesviridae. The family Herpesviridae belongs to the Herpesviridae order. The commonly known human herpesviruses include Epstein–Barr virus (EBV), human cytomegalovirus (HCMV), herpes zoster virus, and herpes simplex virus type 1 and type 2. The anti-herpesvirus ability of artemisinin and its derivatives have been revealed in many researches.

EBV. The human γ-herpesvirus, EBV, is one of the most common herpesviruses. The half-maximal inhibitory concentration (IC50) of artesunate against EBV strain B95-8 *in vitro* was 6.4 ± 2.7 μmol/L in Raji cells (immortalized B lymphocytes), and 3.1 ± 0.9 μmol/L in 293T cells (immortalized epithelial cell type). It is noteworthy that the drug-induced cytotoxicity of artesunate could be excluded by the fact that artesunate did not have remarkable cytotoxic effects on Raji cells in a range between 0.1 μmol/L and 90 μmol/L. The antiviral mode of artesunate is based on preventing the viral immediate-early protein synthesis rather than inhibiting EBV cell adsorption or entry. Therefore, the therapeutic property of artesunate to inhibit ongoing lytic replication makes it superior to many other conventional therapeutic agents.

HCMV. Among the Herpesviridae family, HCMV is the most prevalent virus to which the population is susceptible. Artemisone has significant anti-HCMV activity against laboratory-adapted and low-passage-number clinical strains as well as drug-resistant HCMV strains by inhibiting HCMV replication, and artemisinin could

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000002934

Hao Liu, Zhen Li, and Yang Zhang contributed equally to this work.

Correspondence to: Prof. Caiping Guo, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China
E-Mail: gcp001@ccmu.edu.cn

Chinese Medical Journal 2023;136(24)
Received: 16-06-2023; **Online:** 28-11-2023 **Edited by:** Yanjie Yin

significantly reduce viral mRNA accumulation and completely inhibit viral yield. In addition, the antiviral efficacy of artemisone was superior to that of artesunate with its antiviral activity consistently surpassing that of artesunate ≥10 folds. The effect of artemisone on transcription of the main HCMV immediate-early (IE) genes, and artemisone reduces viral production by inhibiting the expression of HCMV IE1 and IE2 messenger RNA (mRNA).[3]

The antiviral mechanism against HCMV of artemisinin may be manifested in the following aspects. Firstly, artemisinin and its derivatives interfere with viral replication by inhibiting the nuclear factor (NF)-κB pathway. Artemisinin can alkylate the DNA-binding domain of the p65 subunit to inhibit the binding of specificity protein 1 (SP1) and NF-κB, which reduces the activation of very early HCMV promoters and the expression of related genes to suppress viral replication. In addition, artesunate can inhibit the viral nucleocytoplasmic transport recognized by NF-κB (p65) and exportin-1. By preventing p65 from exiting the nucleus, artesunate can reduce the activation of p65 in the cytoplasm, and eventually play a role in inhibiting the expression of related genes and replication of HCMV. Secondly, artemisinin-derived compounds can change the alteration of mitochondrial configuration induced by HCMV infection. There is a significant accumulation of the artemisinin-derived compound BG95 in mitochondria, which has caused dose-dependent changes in the structure of mitochondria. In addition to making the vast majority of cells (>95%) show small, broken dotted phenotypic mitochondria, BG95 also reduced mitochondrial membrane potential, as HCMV replication requires an increase in adenosine triphosphate (ATP) levels, and the reduction of mitochondrial membrane potential can significantly counteract effective virus replication. Thirdly, immunoprecipitation-mass spectrometry (IP-MS) followed by molecular, biological, and biochemical studies verified the type III-intermediate filament protein vimentin as an artemisinin target against HCMV. The binding of artesunate, an artemisinin monomer, to vimentin counteracts virus-induced vimentin degradation and decreases vimentin phosphorylation at Ser-55 and Ser-83, inducing calpain activity. Therefore, artesunate binding to vimentin early during infection stabilizes it and antagonizes subsequent HCMV-mediated vimentin destabilization, effects culminating in virus inhibition.

***Human herpesvirus 6* (*HHV-6*).** HHV-6 is a widespread beta-herpesvirus that exhibits a wide cell tropism *in vivo* and induces a lifelong latent infection in humans. The antiviral activity of artesunate against HHV-6 was demonstrated in a child affected by HHV-6B-associated myocarditis in a report. Conventional treatments for HHV-6B-associated myocarditis were invalid, then the child was administered with artesunate intravenously ($5\ mg{\cdot}kg^{-1}{\cdot}day^{-1}$) for 10 days and continued via oral therapy (2 × 5 mg/kg) for another 10 days. During treatment with artesunate, no side effects were observed, and researchers were surprised to find that artesunate reduced the level of HHV-6B DNA in the myocardium; the patient's clinical status and cardiac function were greatly improved as well. A recent study compared the efficacy of valaciclovir, valganciclovir, and artesunate in reactivated HHV-6 infections. The results showed that negative polymerase chain reaction (PCR) results in patients with HHV-6 treated with valaciclovir were achieved in 26% (first month), 34% (second month), and 37% of cases (third month).[4] The same results with valganciclovir were obtained in 35% (first month), 44% (second month), and 48% of cases (third month), but with artesunate in 44% (first month), 57% (second month), and 68% of cases (third month).[4] Artesunate is more effective than valganciclovir and valacyclovir in patients with reactivated HHV-6 infections.

Flaviviridae. The Flaviviridae belongs to the order Flaviviridae and is a group of enveloped positive-strand RNA viruses with a length of 9.6–12.3 kb. Common ones are the Japanese encephalitis virus (JEV), Zika virus, hepatitis C virus (HCV), and dengue virus.

HCV. The virion of HCV is spherical, less than 80 nm in diameter, and is a single-stranded positive-strand RNA virus. Artesunate inhibited HCV replication without adverse effects on host cells in a concentration and time-dependent manner. Furthermore, a low dose of IFN combined with a low dose of artesunate obtained the same anti-HCV effect as a high dose of IFN alone. HCV replication is accompanied by activation of the nuclear factor E2-related factor 2/antioxidant responsive element (Nrf2/ARE) pathway. Artemisinin combined with heme could enhance the anti-HCV ability, while the anti-HCV capability in combination with the reactive oxygen species inhibitor N-acetylcysteine was significantly reduced. It is speculated that artemisinin and its derivatives will generate reactive oxygen species or carbon-centered radicals after the cleavage of the peroxide bridge of artemisinin. Reactive oxygen species regulate Kelch-like ECH-associated protein 1 (Keap1)/Nrf2/ARE, then reduce the amount of *NS3* and *NS5A* in the replication complex of HCV by disrupting the complex, finally exerting the effect of anti-HCV.

JEV. JEV is an 11-kilobase enveloped positive-sense single-stranded RNA that has five genotypes, all of which could badly affect the central nervous system. In a JEV-infected mouse model, treatment with artemisinin or artesunate reduced its mortality rate from 100% to 50% and 60%, respectively. The astrogliosis, microgliosis, and neuronal cell death in JEV-infected mice had been diminished after the treatment with artemisinin or artesunate. It was found that artemisinin repressed the JEV particle production in a concentration-dependent manner with an IC50 of 18.5 μmol/L *in vitro*. Mechanistically, artemisinin does not block the cell-binding or disrupt the entry ability of the flavivirus, but significantly increases the mRNA expression and secretion of IFN-β and the transcription of interferon-stimulated genes (ISGs), then activates interferon regulatory factor 3 (IRF3) and induces phosphorylation of signal transducers and activators of transcription 1/signal transducers and activators of transcription 2 (STAT1/STAT2) in infected cells.[5]

Human Immunodeficiency Virus (HIV). HIV is a virus that attacks the body's immune system. HIV envelope glycoprotein attaches the virion to a susceptible cell and

induces fusion of viral and cell membranes to initiate infection. It interacts with the primary receptor CD4 and coreceptor to allow viral entry to induce membrane fusion, then it uses reverse transcriptase and integrase to complete its replication. HIV can destroy a large number of CD4 cells, weaken the body's immune function, and cause many fatal complications eventually. Many studies have found that artemisinin and its derivatives may exert anti-HIV activity. *A. annua* tea infusions inhibited HIV with an IC50 value of 2.9 μg/mL *in vitro*. Kiang *et al*[6] reported that the methanol extract of artemisinin could inhibit the activity of HIV-1 protease. A series of artemisinin derivatives with trioxane structure have been synthesized and studied against HIV-1 *in vitro*.[7] Artemisinin showed weak anti-HIV-1 activity based on its unique peroxy bridge structure demonstrated by an increased effective concentration (EC50) and IC50 value in this research. Further investigation of the semisynthetic derivatives revealed that 10-ethoxy decarbonylation artemisinin and high decarbonization artemisinin had moderate anti-HIV-1 activity. Jana *et al*[8] synthesized six 1,5-disubstituted 1,2,3-triazole derivatives of dihydroartemisinin, and three of them showed powerful anti-HIV function with IC50 value is 1.34–2.65 μmol/L. To date, the available *in vitro* data do not support a major role for the leading compound artemisinin in HIV infection control, but its novel derivatives offer a good alternative.

To sum up, artemisinin and its derivatives displayed antiviral effects against various viruses mainly by inhibiting the activation of cellular transcription factors, interfering with the viral replication cycle, inducing cell apoptosis, and preventing the virus from binding to host cells. It needs to be emphasized that convincing clinical evidence for the antiviral effect of artemisinin is still lacking, so researchers should be encouraged to conduct more clinical trials to open new perspectives regarding the use of artemisinin and its derivatives in clinical applications.

Funding

This work was supported by grants from the National Natural Science Foundation of China (Nos. 82072271 and 82072294), the Beijing Natural Science Foundation (No. 7222091), the Peak Talent Program of Beijing Hospital Authority (No. DFL20191701), the Beijing Hospitals Authority Innovation Studio of Young Staff Funding Support (No. 202137), the High-level Public Health Technical Personnel Construction Project, and the Beijing Key Laboratory for HIV/AIDS Research (No. BZ0089).

References

1. Sehailia M, Chemat S. Antimalarial-agent artemisinin and derivatives portray more potent binding to Lys353 and Lys31-binding hotspots of SARS-CoV-2 spike protein than hydroxychloroquine: Potential repurposing of artenimol for COVID-19. J Biomol Struct Dyn 2021;39:6184–6194. doi: 10.1080/07391102.2020.1796809.
2. Li G, Yuan M, Li H, Deng C, Wang Q, Tang Y, *et al*. Safety and efficacy of artemisinin-piperaquine for treatment of COVID-19: An open-label, non-randomised and controlled trial. Int J Antimicrob Agents 2021;57:106216. doi: 10.1016/j.ijantimicag.2020.106216.
3. Oiknine-Djian E, Weisblum Y, Panet A, Wong HN, Haynes RK, Wolf DG. The artemisinin derivative artemisone is a potent inhibitor of human cytomegalovirus replication. Antimicrob Agents Chemother 2018;62:e00288–e18. doi: 10.1128/aac.00288-18.
4. Maltsev D. A comparative study of valaciclovir, valganciclovir, and artesunate efficacy in reactivated HHV-6 and HHV-7 infections associated with chronic fatigue syndrome/myalgic encephalomyelitis. Microbiol Immunol 2022;66:193–199. doi: 10.1111/1348-0421.12966.
5. Wang X, Zheng B, Ashraf U, Zhang H, Cao C, Li Q, *et al*. Artemisinin inhibits the replication of flaviviruses by promoting the type I interferon production. Antiviral Res 2020;179:104810. doi: 10.1016/j.antiviral.2020.104810.
6. Kiang TK, Wilby KJ, Ensom MH. Clinical pharmacokinetic drug interactions associated with artemisinin derivatives and HIV-antivirals. Clin Pharmacokinet 2014;53:141–153. doi: 10.1007/s40262-013-0110-5.
7. D'Alessandro S, Scaccabarozzi D, Signorini L, Perego F, Ilboudo DP, Ferrante P, *et al*. The use of antimalarial drugs against viral infection. Microorganisms 2020;8:85. doi: 10.3390/microorganisms8010085.
8. Jana S, Iram S, Thomas J, Hayat MQ, Pannecouque C, Dehaen W. Application of the triazolization reaction to afford dihydroartemisinin derivatives with anti-HIV activity. Molecules 2017;22:303. doi: 10.3390/molecules22020303.

How to cite this article: Liu H, Li Z, Zhang Y, Jia L, Cai MT, Wang R, Guo CP. Antiviral effects of artemisinin and its derivatives. Chin Med J 2023;136:2993–2995. doi: 10.1097/CM9.0000000000002934