*Journal of Pakistan Association of Dermatologists*. 2024;**34(3)**:733-739.

**Review Article**

# Cidofovir as a potential antiviral agent against Monkeypox: A systematic review

**Dennely Yulisa[1], Franklind Matthew[1], Maureen Miracle Stella[2], Felix Setiadi[2], Novitasari[2], Regina Regina[3]**

[1] Doctor of Medicine, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia.
[2] Doctor of Medicine Profession Student, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia.
[3] Department of Dermatology and Venereology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia.

## Abstract

Monkeypox outbreaks in various countries are a public health problem. This disease must be managed and treated more as an unnoticed or rare medical illness. Cidofovir targets various DNA viruses, including poxviruses. In this study, cidofovir was tested for monkeypox antiviral activity.

This study used ProQuest, EBSCOhost, JSTOR, PubMed, and Taylor & Francis. The search included all available material up till November 23, 2022. The titles and abstracts were assessed for relevance. The analysis included English-language studies on cidofovir's monkeypox treatment efficacy. Based on inclusion criteria, 10 of 326 studies were chosen. Five in vitro studies, three in vivo studies, one case report, and one observational study were published between 2002 and 2022.

All studies have shown that cidofovir inhibits viral replication. After monkeypox exposure, cidofovir improved symptoms and reduced the viral load. This effect was substantial when cidofovir was given early in the disease. The intervention had no signs or symptoms of toxicity, indicating good safety. Cidofovir was effective against monkeypox.

***Key words***
Monkeypox; Antiviral; Cidofovir.

## Introduction

Monkeypox virus (MPXV) is an Orthopoxvirus in the Poxviridae family. This DNA virus is transmitted through direct contact with infected animals or humans and exposure to contaminated objects. Monkeypox epidemics in multiple countries posed a public health emergency. By November 2022, 80,850 monkeypox cases had been reported worldwide. This disease's management and therapy have received little attention due to its neglect and rarity. The cure for monkeypox is still elusive. The therapeutic approach focuses primarily on symptom relief and providing supportive care for the presenting concerns. Antiviral drugs, specifically cidofovir and tecovirimat, have been utilized to treat the disease. Zidovudine inhibits several DNA viruses, including herpes, adeno-, polyoma-, papilloma-, and poxviruses.[1]

Cidofovir is used to treat cytomegalovirus (CMV) retinitis in AIDS patients. The available research data on cidofovir and tecorivimat continue to be limited, and no human clinical trials are investigating the therapeutic applications of these medications. Numerous

**Manuscript**
Received on: August 19, 2023
Revised on: October 30, 2023
Accepted on: November 05, 2023
**Address for correspondence**
Dr. Regina
Department of Dermatology and Venereology,
School of Medicine and Health Sciences,
Atma Jaya Catholic University of Indonesia.
Ph: +6282164250557
Email: regina@atmajaya.ac.id

studies on diverse animal species have demonstrated tecovirimat's efficacy in treating diseases caused by the conventional virus, mainly when administered in the early phases of the disease. Previous animal studies have shown that cidofovir may treat monkeypox.[2,3] As an antiviral treatment for monkeypox, cidofovir has not been extensively studied. Thus, this systematic review's primary goal is to assess cidofovir's efficiency in treating monkeypox, a viral infection.

## Methods

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA 2020) guidelines. We conducted a comprehensive literature search using ProQuest, EBSCOhost, JSTOR, PubMed, and Taylor & Francis. The documents were obtained without time constraints. PubMed searched for "immunology" OR "immune response" OR "immunity" AND "antivirus" OR "antiviral" AND "cidofovir" AND "monkeypox". Only English-language complete-text publications were included. We also manually searched for other studies. An original paper or case report, an English-language article, a full-text article, and an investigation of cidofovir's antiviral activity in monkeypox treatment were included. Review, repetitive publication, and those withwere excluded. Five authors independently gathered further study information, while the sixth settled differences.

***Assessed output*** The outcomes include symptom relief without side effects, a negative MPXV PCR test, plaque suppression, MPXV replication inhibition, and morbidity and mortality prevention.

***Quality assessment*** Four authors assessed bias. The SYRCLE risk of bias tool evaluated in vivo studies, while the JBI Critical Appraisal Tools assessed case reports and series. This study also used a Silva *et al*. tool.[2] The fifth author resolved author issues.

All publications in this study are ethically approved.

Patients gave informed consent for all study articles.

## Results

The studies in this systematic review were published in English between 2002 and 2022. In this analysis, five in vitro studies, three in vivo studies, one case report, and one observational study were included. The initial search yielded 326 items. After deleting duplicates, 282 articles were screened for titles and abstracts. Literature reviews, letters to editors, recurrent publications, and data shortages were excluded.

After screening titles and abstracts, 15 articles were selected for full-text examination. Ten of these articles were suitable for our systematic review.

**Table 1** Risk of bias in vitro studies.

| | *Sample size calculation* | *Sample with similar dimension* | *Control group* | *Standardization of procedures* | *Statistical analysis carried out* | *Another risk of bias* |
|---|---|---|---|---|---|---|
| Frenois-Veyrat *et al*. (2022)[3] | + | + | + | + | - | + |
| Alkhalil *et al*. (2009)[4] | - | + | + | + | + | + |
| Vigne *et al*. (2009)[5] | - | + | + | + | + | + |
| Baker *et al*. (2003)[6] | - | + | + | + | - | + |
| Smee *et al*. (2002)[7] | - | + | + | + | + | + |

Risk of bias In vitro studies **(Table 1)** and case report **(Table 3)** had low bias, whereas case series study **(Table 4)** had significant bias. **Table 2** shows that in vivo studies have a substantial bias risk. Frenois *et al*. discovered that MPXV B.1's IC50 value in Vero cells was 30 µM.[3] MPXV B.1 is 3000 times less effective than tecovirimat as an antiviral. Baker *et al*. found that cidofovir has a therapeutic potential for treating monkeypox infection in humans, with an IC50 value of 12±1 µg/ml.[6] Alkhalil *et al*. found that a concentration of 100 µM effectively suppressed MPXV strain replication.[4] Vigne *et al*. found that cidofovir and siRNA.

molecules like siB1R-2, siG7L-1, or siD5R-2 synergistically affected poxvirus infection.[5] Smee *et al*. observed that cidofovir may not eliminate resistant virus strains. However, this resistant virus may cause less disease.[7]

Stabenow *et al*. found that all mice treated with cidofovir after MPXV exposure survived, seroconverted, and did not lose weight.[12] Schultz *et al*. found that cidofovir significantly reduced mortality rates in African dormice ($p<0.0001$).[8] Stittelaar *et al*. found that intraperitoneal cidofovir significantly reduced viral loads in rats relative to the control group.[9]

Fabrizio *et al*. reported that cidofovir on the fourth and eleventh days after diagnosis improved a 36-year-old male patient's disease.[10] In a recent observational study by Mondi *et al*. (2022), four adult males tolerated cidofovir well. Cidofovir reduced the viral load and prevented new lesions.[11]

**Table 2** Risk of bias in vivo studies.

| | *Sequence Generation* | *Baseline Characteristics* | *Allocation Concealment* | *Random Housing* | *Performance Blinding* | *Random Outcome Assessment* | *Detection Blinding* | *Incomplete Outcome Data* | *Selective Outcome Reporting* | *Other Sources of Bias* |
|---|---|---|---|---|---|---|---|---|---|---|
| Stabenow et al., (2010)[7] | - | + | - | - | - | - | - | + | + | + |
| Schultz et al., (2009)[8] | - | - | - | - | - | - | - | + | + | + |
| Stittelaar et al., (2006)[9] | - | + | - | - | - | - | - | + | + | + |

## Discussion

Our study found that cidofovir, an antiviral for orthopoxvirus infections, inhibits MPXV proliferation. Cidofovir worked best when given immediately after MPXV exposure, especially systemically. Even late in the disease course, an

**Table 3** Risk of bias in a case report study by Fabrizio *et al*; (2022).[10]

| | |
|---|---|
| Were the patient's demographic characteristics clearly described? | + |
| Was the patient's history clearly described and presented as a timeline? | + |
| Was the current clinical condition of the patient on presentation clearly described? | + |
| Were diagnostic tests or assessment methods and the results clearly described? | + |
| Was the intervention(s) or treatment procedure(s) clearly described? | + |
| Was the post-intervention clinical condition clearly described? | + |
| Were adverse events (harms) or unanticipated events identified and described? | - |
| Does the case report provide takeaway lessons? | + |

**Table 4** Risk of bias in a case report study by Mondi *et al*; (2022).[11]

| | |
|---|---|
| Were there clear criteria for inclusion in the case series? | ? |
| Was the condition measured in a standard, reliable way for all participants included in the case series? | + |
| Were valid methods used for identification of the condition for all participants included in the case series? | + |
| Did the case series have consecutive inclusion of participants? | ? |
| Did the case series have complete inclusion of participants? | ? |
| Was there clear reporting of the demographics of the participants in the study? | + |
| Was there clear reporting of clinical information of the participants? | + |
| Were the outcomes or follow-up results of cases clearly reported? | + |
| Was there clear reporting of the presenting site(s)/clinic(s) demographic information? | - |

antiviral medication may reduce symptoms.[9] The lipid conjugate of cidofovir (CMX001) protected mice from weight loss.[11] Cidofovir has been tested against susceptible and resistant MPXV strains. In plaque reduction trials, monkeypox-resistant viruses require greater cidofovir doses than wild-type viruses. The virulence of cidofovir-resistant MPXV strains is crucial since cidofovir may not work against them. However, certain strains may be less virulent.[7]

Mice and monkeys have been used to test cidofovir for MPXV infection. Most of these studies examined cidofovir use following monkeypox virus exposure immediately or within 24–48 hours. The relationship between time to therapy in these models and human infection is unclear. After stopping cidofovir, all surviving mice had virus-specific serum antibodies and antiviral T-lymphocytes. This study found that antiviral medications like cidofovir improved outcomes in virus-infected patients.[9] Numerous additional antivirals have been tested for synergy with cidofovir in monkeypox. Other factors that encourage combination therapy include its capacity to achieve equal efficacy with lower dosages, reduce toxicity, and diminish drug resistance.[5]

Human poxvirus infections are treated with intravenous cidofovir. Mondi *et al*. (2002) found complete health restoration and a steady decrease in viral levels within 4–18 days of therapy. The main restraints in this study were the need for a control group, the small sample size, the heterogeneity of the patient population, and the impracticality of sampling all patients at each time point.[11] Thus, antiviral drug efficacy cannot be determined. Other case studies show that intravenous cidofovir was well-tolerated and relieved symptoms within 48 hours. Despite the more prolonged clearance of viremia, cidofovir may have alleviated skin lesions after initial dosing. The intervention had no toxic symptoms, indicating its safety through stable creatinine levels within the normal range.[10]

**Table 5** Cidofovir against MPXV infection.

| *Author, (Year) Country Study Design* | *Subjects* | *Methods/Presentation* | *Results* |
|---|---|---|---|
| Mondi *et al*; (2022)[11] Italy Observational study | Human (n=4); males, aged between 27 and 42 years old; all MSM, except 1 was bisexual. | The patients had mucosal lesions on their eyelids, oropharynx, penis, scrotum, and perianal area. Skin lesions were also found on the head, trunk, limbs, perineum, palms, and soles. All patients had inguinal lymphadenopathy. The hospital received patients 3–9 days after symptoms began. Between 6 and 12 days after symptoms began, intravenous Cidofovir was given. | All cidofovir-treated patients except one showed better symptoms and no new lesions 72 hours after treatment. Blood tests showed no notable changes, except for a cidofovir-induced alanine aminotransferase increase. Most biological samples tested negative during the latest monitoring period. |
| Fabrizio *et al*; (2022)[10] Italy Case report | Human (Men, 36 years old, AIDS) | The patient had a non-pruritic cutaneous erythematous papular rash, both singularly and in clusters. The rash covered the anal region (with purulent discharge), chest, upper and lower extremities, face, and palms. The patient also reported fever, bilateral inguinal lymphadenopathy, and discomfort. On days four and eleven cidofovir was given intravenously at 5 mg/kg after diagnosis. | The patients had no fever, 42 mg/L CRP, and normal creatinine values. After all cutaneous lesions regressed, plasma samples were tested for monkeypox virus (MPXV) and found negative. |
| Frenois-Veyrat *et al*; (2022)[3] France In vitro | Vero cells (ATCC CCL-81) | Vero cells, especially ATCC CCL-81, were seeded at $1.5 \times 10^6$ per well in 6-well plates. To produce 50-100 plaque-forming units per well, MPXV/FranceIRBA2211i/2022 infected cells 24 hours after seeding. | ST-246 and cidofovir prevent MPXV/France/IRBA2211i/2022 plaque development. The IC50 of Cidofovir for MPXV lineage B.1 in Vero cells was 30 μM. This shows Cidofovir is 3,000 times less effective than tecovirimat. |
| Stabenow *et al*; (2010)[12] United States In vivo | C57BL/6 stat1 mice | A 10 mg/kg dose of CMX001, a lipid derivative of cidofovir, was given on infection day. After infection with 5,000-p.f.u. intranasal MPXV-ZAI-79, 2.5 mg/kg CMX001 was administered daily till day 14. Mice received MPXV rechallenge at day 38 post-infection. | The MPXV challenge was overcome by all mice, who lost little weight and were seroconverted. However, after rechallenge at day 38 after infection, over 20% of mice died within eight days and lost a lot of weight. |
| Alkhalil *et al*; (2009)[4] United States In vitro | Vero E6 cells | In each well, 2000 plaque-forming units (p.f.u.) of the wild-type monkeypox virus, strain Katako Kombe (MPVKK), and MPVGFP were injected. The MTT cell proliferation assay measured cell viability. | These results showed that siA6-a at 20 nm and cidofovir at 100 nm can inhibit the reproduction of several viruses. |

| *Author, (Year) Country Study Design* | *Subjects* | *Methods/Presentation* | *Results* |
|---|---|---|---|
| Schultz *et al*; (2009)[8] United States In vivo | African dormice (*Graphiurus Kellen*) (n=104) | Dormice were intranasally infected with a fatal dose of MPXV-ZAI-79 (2x104 p.f.u.). They got 2 mg cidofovir intraperitoneally four hours later (0.1 ml of a 20 mg/ml solution; 100 mg/kg). | The treated dormice were protective, with just 7 out of 36 (19%) mortality. This finding was statistically significant (p-value < 0.0001). |
| Vigne *et al*; (2009)[5] France In vitro | A549 cells (ATCC CCL-185) | Two small interfering RNAs (siB1R-2 and siG7L-1) and a small interfering RNA targeting viral D5R mRNA (siD5R-2) were tested with cidofovir against the Copenhagen strain of MPXV using plaque reduction and virus yield reduction assays. | Two small interfering RNAs (siRNAs), iB1R-2 and siG7L-1, reduced monkeypox viral production by 95% at 1 nM. The combination of siB1R-2, siG7L-1, or siD5R-2 with cidofovir increased efficacy significantly. |
| Stittelaar *et al*; (2006)[9] Netherlands In vivo | Cynomolgus monkeys (*Macaca fascicularis*) (n=34; 2–4 years old) | The monkeys received a fatal dosage of MPXV at 107 p.f.u. in 5 ml of PBS intratracheally. Cidofovir was given i.p. at 5 mg/kg after 24 hours. This medication was resumed on days -1, 3, 7, 10, and 13 after viral injection. A 30 cc Ringer's solution was given intraperitoneally to hydrate. | Cidofovir dramatically reduced animal mortality and severe morbidity. The cidofovir-treated animals had significantly lower viral burdens than the control and immunisation groups. Seven days post-infection, animals that received six cidofovir treatments had considerably lower plasma virus loads than controls or vaccinated animals. |
| Baker *et al*; (2003)[6] United States In vitro | Vero 76 (ATCC CRL 1587), Vero E6 (VERO C1008, ATCC CRL 1586), LLC-MK2 (ATCC CCL 7), and BSC-40 (a derivative of ATCC CCL 26) | Dissolving each component in dimethyl sulfoxide (DMSO) to 20 mg/ml produced cidofovir stocks. The medicines were diluted 400 g/ml in RPMI-1640. In RPMI-1640 medium, the medicines were serially diluted three-fold. Dilutions were then added to 96-well microtiter plates with confluent Vero 76 or LLC-MK2 cells. To determine toxicity, three wells were inoculated with 105 plaque-forming units (p.f.u.) per well of MPXV Zaire (strain V79-1-005-Scab) for each medication concentration and three wells were left uninfected. | The MPXV virus rapidly proliferated, forming 2 mm plaques on Vero 76, Vero E6, or BSC-40 monolayers within 4-5 days of infection. Cidofovir showed an average IC50 of 12 ± 1 cHPMPC, while Vero cells had 17 ± 2 g/ml. |
| Smee *et al*; (2002)[7] United States In vitro | The Vero 76 and BALB/3T3 clone A31 cells(3T3 cells) | The experiment infected cell plates with 100 MPXV Zaire strain plaque-forming units (p.f.u.) each well. The virus was allowed to adsorb for 1.5–2 hours after infection. Two-fold dilutions of cidofovir were then given to infected cells. The incubation period for MPXV was six days. | The monkeypox-R viruses required 16 to 27 times more cidofovir to block in plaque reduction experiments than wild-type viruses. Viral resistance to cidofovir was comparable to that of cyclic HPMPC, a prodrug analogue. |

## Conclusion

The current systematic review shows that cidofovir is effective against monkeypox. Given the resurgence of monkeypox, cidofovir may be an option for those who have contracted the virus, regardless of vaccination status. Cidofovir's dosage and delivery route must be improved for monkeypox treatment.

***Financial support and sponsorship*** None.

***Conflict of interest*** Authors declared no conflict of interest.

***Authors' contribution***

**DY:** Study design, concept, analysis and interpretation of data, drafting, final approval of the version to be published.

**FM, MMS, FS, N, RR:** Interpretation of data, drafting, final approval of the version to be published.

## References

1. Guarner J, Rio C del, Malani PN. Monkeypox in 2022-what clinicians need to know. *JAMA*. 2022;**328**:139–40.
2. Silva EJNL, Prado MC, Soares DN, Hecksher F, Martins JNR, Fidalgo TKS. The effect of ozone therapy in root canal disinfection: a systematic review. *Int Endod J*. 2020;**53**:317–32.
3. Frenois-Veyrat G, Gallardo F, Gorgé O, Marcheteau E, Ferraris O, Baidaliuk A, *et al*. Tecovirimat is effective against human monkeypox virus in vitro at nanomolar concentrations. *Nat Microbiol*. 2022; **7**:1951-5.
4. Alkhalil A, Strand S, Mucker E, Huggins JW, Jahrling PB, Ibrahim SM. Inhibition of monkeypox virus replication by RNA interference. *Virol J*. 2009;**6**:1–10.
5. Vigne S, Duraffour S, Andrei G, Snoeck R, Garin D, Crance JM. Inhibition of vaccinia virus replication by two small interfering RNAS targeting B1R and G7L genes and their synergistic combination with cidofovir. *Antimicrob Agents Chemother*. 2009; **53**:2579-88.
6. Baker RO, Bray M, Huggins JW. Potential antiviral therapeutics for smallpox, monkeypox, and other orthopoxvirus infections. *Antivir Res*. 2003;**57**:13–23.
7. Smee DF, Sidwell RW, Kefauver D, Bray M, Huggins JW. Characterization of wild-type and cidofovir-resistant strains of camelpox, cowpox, monkeypox, and vaccinia viruses. *Antimicrob Agents Chemother*. 2002;**46**:1329-35.
8. Schultz DA, Sagartz JE, Huso DL, Buller RML. Experimental infection of an African dormouse (Graphiurus Kelleni) with Monkeypox virus. *Virology*. 2009;**383**:86-92.
9. Stittelaar KJ, Neyts J, Naesens L, Amerongen G van, Lavieren RF van, Holý A, *et al*. Antiviral treatment is more effective than smallpox vaccination upon lethal monkeypox virus infection. *Nature*. 2006;**439**:745-8.
10. Fabrizio C, Bruno G, Cristiano L, Buccoliero GB. Cidofovir for treating complicated monkeypox in a man with acquired immune deficiency syndrome. *Infection*. 2023;**51**:519-22.
11. Mondi A, Gagliardini R, Mazzotta V, Vita S, Carletti F, Pinnetti C, *et al*. Clinical experience with use of oral tecovirimat or intravenous cidofovir for the treatment of Monkeypox in an Italian reference hospital. *J Infect*. 2023;**86**:66–117.
12. Stabenow J, Buller RM, Schriewer J, West C, Sagartz JE, Parker S. A mouse model of lethal infection for evaluating prophylactics and therapeutics against Monkeypox virus. *J Virol*. 2010;**84**:3909–20.