Active cytomegalovirus particles in the eyes of an AIDS patient being treated with 9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl] guanine (Ganciclovir)

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SUMMARY The eyes of an AIDS patient with cytomegalovirus (CMV) retinitis and pneumonitis who died while receiving maintenance therapy with the antiviral agent 9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl] guanine (Ganciclovir) were obtained for pathological examination. While under treatment the patient had significant improvement but not complete regression of retinitis. Electron microscopic and immunofluorescent techniques revealed cytomegalovirus particles in the retina, sclera, iris, and ciliary body. These findings are consistent with a virostatic type of inhibition of CMV by this agent. They also suggest that CMV involvement in the eye and other organs may be more widespread than is clinically apparent in AIDS patients.

Cytomegalovirus (CMV) infections occur commonly in patients with the acquired immunodeficiency syndrome (AIDS) and represent an important cause of morbidity and mortality in these patients.^{1–3} The retina is frequently involved by CMV,^{4–7} and retinitis may be the first clinical sign of disseminated infection with this organism.⁸ Untreated CMV retinitis in AIDS patients relentlessly progresses to involve the entire retina, resulting in irreversible blindness.⁵ Recently a new antiviral agent 9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl] guanine (Ganciclovir) or BWB759U (Burroughs Wellcome Co., Research Triangle Park, North Carolina), has been shown to effect at least stabilisation or partial improvement of CMV retinitis in over 80% of the affected eyes in immunosuppressed patients treated with this drug.^{9–12} Unfortunately, the vast majority of these patients with AIDS have relapses within two to six weeks of discontinuing therapy.^{9–14} This has led some investigators to believe that long-term intravenous therapy will probably be necessary.^{9–14} We report here the clinical course and histopathological and ultrastructural findings in the eyes of an AIDS patient

with CMV retinitis and pneumonitis who died while receiving maintenance intravenous therapy with Ganciclovir.

Case report

A 30-year-old homosexual white male was admitted to Beth Israel Medical Center 4 September 1985 for an episode of dysphasia and dizziness lasting 4–5 hours. AIDS had been diagnosed in November 1984 when he developed *Pneumocystis carinii* pneumonia. For three months prior to admission he had persistent diarrhoea due to biopsy-proved cytomegalovirus (CMV) colitis. He had also developed generalised weakness of his arms and legs for six weeks before admission due to an AIDS-related peripheral neuropathy.

On admission the patient had no visual complaints, but an ophthalmic consultation was requested because of a cotton-wool spot noted in the left eye. An ophthalmic examination of 6 September 1985 revealed a best corrected vision of 20/25 in each eye. Ocular motility, confrontation visual fields, and pupillary reactions were normal, with no afferent pupillary defect. Slit-lamp examination showed normal anterior segments except for dilated seg-

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ments of conjunctival venules near the nasal limbus OU. Ophthalmoscopic examinations, with dilated pupils, of the fundi with direct and indirect ophthalmoscopy showed an area of yellowish perivascular opacification in the far inferotemporal retina of the right eye without evidence of vitreous inflammation. This area was 2.5 disc diameters in size and was consistent clinically with CMV retinitis. A cotton-wool spot was present along the inferotemporal arcade of the left eye. No other fundus abnormalities were noted.

A serum CMV enzyme-linked immunosorbent assay (ELISA) was equal to 0.67. A toxoplasmosis titre was negative. Serum and urine cultures for CMV were negative. A lumbar puncture did not reveal any cells or organisms in the cerebrospinal fluid (CSF), and all cultures including viral cultures of the CSF were negative.

The patient was discharged after five days without determination of a cause for his acute neurological symptoms, which did not recur.

He was readmitted 10 October 1985 because of progressing limb weakness, fever to 39.4°C, and diffuse interstitial pulmonary infiltrates on his chest x-ray. He still appeared to be asymptomatic. An ophthalmic examination on 15 October 1985 showed the best corrected visual acuity to be 20/25 OU. Ophthalmoscopy of the right eye showed the retinitis to have progressed along the inferotemporal vessels to within 2 disc diameters of the inferotemporal arcade. No retinitis was seen in the left eye. An endobronchial biopsy on 17 October 1985 revealed *Pneumocystis carinii* organisms, and culture of the biopsy material subsequently grew cytomegalovirus. Because it was thought that visual loss in the right eye was imminent, the patient was given intravenous pentamidine, and treatment with Ganciclovir (supplied by the Burroughs Wellcome Co. on a compassionate plea protocol) was begun on 17 October 1985 at a dosage of 2.5 mg/kg intravenously every eight hours. A white blood cell count (WBC) at this time was $13.2 \times 10^9/l$ and haemoglobin (Hb) was 9.6 g/dl. Over the next 24 hours there was a slight extension of the retinitis in the right eye, which then stabilised. By the 13th day of treatment partial fading of the area of retinitis in the right eye was observed, with no change in visual acuity. He was afebrile, with less diarrhoea and improvement of the pulmonary infiltrates on chest x-ray. On the 21st day of treatment vision was still 20/25 OU, with marked improvement in the retinitis of the right eye. The initial area of acute retinitis was converted into a granular pigmented chorioretinal scar with a thin surrounding rim of residual retinitis (Fig. 1). The same dosage of Ganciclovir was continued until his discharge from the hospital on 13 November 1985 for

Fig. 1 Fundus photograph of right eye taken 22 days after beginning therapy with Ganciclovir demonstrating residual CMV retinitis around the edges of chorioretinal scarring just outside the inferotemporal arcade.

a total of 28 days. At this time his vision was unchanged and the residual rim of retinitis in the right eye was fainter. He remained afebrile, his pulmonary infiltrates had cleared, and the WBC was $4.7 \times 10^9/l$. Serum and urine cultures for CMV were negative.

On leaving the hospital the patient was put on a home maintenance programme of Ganciclovir at a dosage of 5 mg/kg intravenously once daily for five days per week.

He was examined again on 21 November 1985, when he had no visual complaints and his vision was still 20/25 OU. The residual retinitis in the right eye was less prominent round the chorioretinal scar, but two spots of retinal opacification each about $\frac{1}{3}$ of a disc diameter were seen in the temporal peripheral retina of the left eye, suggestive of early foci of retinitis.

On 5 December 1985 his vision was unchanged, but there was a slight increase in the density and width of the area of retinitis surrounding the chorioretinal scar inferotemporal to the posterior pole of the right eye. There was no change in the left eye. The WBC was now $4 \times 10^9/l$. The home dosage of Ganciclovir was increased to 5 mg/kg each morning and 2.5 mg/kg each evening every day. This was continued for 21 days, and on 26 December 1985 his vision was still 20/25 OU, with no change in the retinitis of either eye. The dosage of Ganciclovir was then reduced to 5 mg/kg once a day for seven days a week. His

Fig. 2 Photomicrograph of retina at level of the equator showing an area of retinal necrosis (arrow) and retinal pigment epithelial degeneration. Haematoxylin and eosin. $\times 216$.

WBC during this time fluctuated between 2.4 and $3.0 \times 10^9/l$.

The patient was again admitted to hospital on 29 December 1985 for bilateral pneumonia with a right sided pleural effusion after 46 days of home maintenance Ganciclovir. His WBC at this time was $5.5 \times 10^9/l$. An ophthalmic examination on 30 December 1985 showed his vision to still be 20/25 OU, with no change in the retinitis of either eye—neither regression nor progression. He was maintained on 5 mg/kg Ganciclovir daily. Blood and pleural fluid cultures grew *Staphylococcus aureus*. All cultures were negative for cytomegalovirus. Despite treatment with appropriate antibiotics the patient died from respiratory arrest on 2 January 1986 after a total of 76 days of treatment with

Ganciclovir. Consent was obtained for post-mortem removal of the eyes.

PATHOLOGICAL EXAMINATION

Gross examination of the right eye revealed an intact globe measuring $25 \times 24.5 \times 24.5$ mm with 8 mm of attached optic nerve. The globe transilluminated normally and was opened through the plane of retinal involvement that had been described clinically. There were several intraretinal foci of whitish infiltration with associated perivascular cuffing. The optic nerve appeared normal.

Microscopic examination of the right eye revealed a normal anterior segment. The vitreous was clear. Areas of peripheral retina showed perivascular cuffing by neutrophils and rare lymphocytes. An atrophic area of retina was noted just anterior to the equator (Fig. 2). The retinal pigment epithelium here showed a mild disturbance (Fig. 2). There was a prominent area of superficial retinal necrosis which contained cells containing small intracytoplasmic inclusions as well as larger cells with intranuclear 'owl's eye' inclusions (Fig. 3A). Immunoperoxidase stained serial sections demonstrated products specific for cytomegalovirus both in the central necrotic zone and surrounding cells (Fig. 3B). Similarly stained sections specific for CMV revealed virus in the anterior iris stroma (Fig. 4A), surrounding iris vessels, and in the stroma of the ciliary processes (Fig. 4B). Electron micrographs of the retina revealed both typical hexagonally shaped capsomers of herpesvirus (family) and larger more electron-dense forms associated more specifically with cytomegalovirus (Fig. 5).¹⁵

Fig. 3A Photomicrograph of retina showing a focal area of necrosis. Note cells with viral particles in the cytoplasm (arrow). Haematoxylin and eosin. $\times 750$.

Fig. 3B Immunoperoxidase stain specific for CMV demonstrates viral material in retina (arrow). $\times 750$.

Fig. 4A Immunoperoxidase stain of iris shows viral material (arrow) in superficial iris (control negative). $\times 790$.

Discussion

The presence of viral particles suggestive of CMV in the retina of this patient is not surprising, since there was clinically residual retinitis at the time of death. The progression of retinitis, however, was minimal during the time of treatment with Ganciclovir. This is consistent with studies that have demonstrated virostatic reversible-type inhibition of CMV by Ganciclovir.^{16,17} The mode of action of Ganciclovir is similar to that of acyclovir in that both are phosphorylated by herpes thymidine kinase and inhibit viral DNA synthesis more than that of the host cell.¹⁸ Ganciclovir is markedly more active than acyclovir against human CMV.^{18,19} However, the mechanism of action of Ganciclovir against human CMV has not been fully elucidated. On removal of Ganciclovir, DNA synthesis in CMV resumes and infectious virus reappears.^{16,17} Of interest is the demonstration via immunofluorescent techniques of CMV capsular proteins in the sclera, iris, and ciliary body of this patient despite a lack of clinical evidence of disease in these structures. There may not necessarily have been infectious virus in these tissues, since there is greater resistance of viral polypeptide synthesis than of either infectivity or viral DNA synthesis to inhibi-

Fig. 4B Stroma of ciliary processes stain positively with immunoperoxidase (control negative). $\times 200$.

tion by Ganciclovir.^{16,17,20} However, CMV has been found on electron microscopic evaluation of the conjunctiva of an AIDS patient who had no clinical evidence of conjunctivitis.²¹ This suggests that CMV involvement in the eye and other organs may be more widespread than is clinically apparent in AIDS patients.

Although Ganciclovir was unable to eliminate CMV from the retina and other ocular structures, this patient still appeared to benefit from treatment. He maintained useful vision in both eyes and was relatively visually asymptomatic until his death. He also had clinical improvement in his CMV pneumonitis and colitis.

The virostatic effect of Ganciclovir on CMV shown in this patient and in other studies⁹⁻¹⁴ implies that AIDS patients with CMV infections may need to be maintained on this drug indefinitely and that the maintenance dosage should be tailored to the individual. It is conceivable that his CMV retinitis might have regressed further if Ganciclovir had been continued at the higher dosage of 7.5 mg/kg per day. On the other hand drug toxicity, most notably leucopenia,⁹⁻¹² may be more common and more severe at higher dosages. This patient did indeed have a lowering of his WBC on the higher dosage. A controlled trial is needed to establish optimum maintenance regimens and to elucidate more fully the efficacy and safety of this drug in AIDS patients. The development of agents not requiring long-term maintenance and of lower toxicity should be sought.

Fig. 5 Electron micrograph of clinically involved retina reveals hexagonally shaped capsomers of the herpesvirus type. Incomplete forms devoid of capsomers which appear as empty circles. Inset (top left) depicts larger more electron-dense forms consistent with cytomegalovirus within infected cells. $\times 57\,500$.

The 9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl] guanine was supplied by Donna Cederberg of Burroughs Wellcome Co., Research Triangle Park, North Carolina.

References

- 1 Quinnan CV, Masur H, Rook AH, *et al.* Herpesvirus infections in the acquired immune deficiency syndrome. *JAMA* 1984; **252**: 72-7.
- 2 Welch K, Finkbeiner W, Alpers CE, *et al.* Autopsy findings in the acquired immune deficiency syndrome. *JAMA* 1984; **252**: 1152-9.
- 3 Macher AM, Reichert CM, Straus SE, *et al.* Death in the AIDS patient: role of cytomegalovirus. *N Engl J Med* 1983; **309**: 1454.
- 4 Holland GN, Pepose JS, Thomas HP, *et al.* Acquired immune deficiency syndrome: ocular manifestations. *Ophthalmology* 1983; **90**: 859-73.
- 5 Palestine AG, Rodrigues MM, Macher AM, *et al.* Ophthalmic involvement in acquired immunodeficiency syndrome. *Ophthalmology* 1984; **91**: 1092-9.
- 6 Pepose JS, Holland GN, Nestor MS, *et al.* Acquired immune deficiency syndrome: pathogenic mechanisms of ocular disease. *Ophthalmology* 1985; **92**: 472-84.
- 7 Reichert CM, O'Leary TJ, Levens DL, Simrell CR, Macher AM. Autopsy pathology in the acquired immune deficiency syndrome. *Am J Pathol* 1983; **112**: 357-82.
- 8 Murray HW, Knox DL, Green WR, Susel RM. Cytomegalovirus retinitis in adults, a manifestation of disseminated viral infection. *Am J Med* 1977; **63**: 574-84.
- 9 Palestine AG, Stevens G, Lane HC, *et al.* Treatment of cytomegalovirus retinitis with dihydroxy propoxymethyl guanine. *Am J Ophthalmol* 1986; **101**: 95-101.
- 10 Masur H, Lane HC, Palestine A, *et al.* Effect of 9-(1,3 dihydroxy-2-propoxymethyl) guanine on serious cytomegalo-

- virus disease in eight immunosuppressed homosexual men. *Ann Intern Med* 1986; **104**: 41-4.
- 11 Collaborative DHPG Treatment Study Group. Treatment of serious cytomegalovirus infections with 9-(1,3-dihydroxy-2-propoxymethyl) guanine in patients with AIDS and other immunodeficiencies. *N Engl J Med* 1986; **314**: 801-5.
- 12 Rosecan LR, Stahl-Bayliss CM, Kalman CM, Laskin OL. Antiviral therapy for cytomegalovirus retinitis in AIDS with dihydroxy propoxymethyl guanine. *Am J Ophthalmol* 1986; **101**: 405-18.
- 13 Felsenstein D, D'Amico DJ, Hirsch MS, *et al*. Treatment of cytomegalovirus retinitis with 9-[2-hydroxy-1-(hydroxymethyl) ethoxymethyl] guanine. *Ann Intern Med* 1985; **103**: 377-80.
- 14 Bach MC, Bagwell SP, Knapp NP, Davis KM, Hedstrom PS. 9-(1,3 dihydroxy-2-propoxymethyl) guanine for cytomegalovirus infections in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1985; **103**: 381-2.
- 15 Chumbley LC, Robertson DM, Smith TF, Campbell RJ. Adult cytomegalovirus inclusion retino-uveitis. *Am J Ophthalmol* 1975; **80**: 807-16.
- 16 Mar E-C, Cheng Y-C, Huane E-S. Effect of 9-(1,3 dihydroxy-2-propoxymethyl) guanine on human cytomegalovirus replication in vivo. *Antimicrob Agents Chemother* 1983; **24**: 518-21.
- 17 Tocci MJ, Livelli TJ, Perry HC, Crumpacker CS, Field AK. Effect of the nucleoside analog 2'-nor-2'-deoxyguanosine on human cytomegalovirus replication. *Antimicrob Agents Chemother* 1984; **25**: 247-52.
- 18 Smee DP, Martin JC, Verheyden JPH, Matthews TR. Anti-herpesvirus activity of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl) guanine. *Antimicrob Agents Chemother* 1983; **23**: 676-82.
- 19 Tyms AS, Davis JM, Jeffries DJ, Meyers JD. BWB759U: an analogue of acyclovir, inhibits human cytomegalovirus *in vitro*. *Lancet* 1984; *ii*: 924-5.
- 20 Rasmussen L, Chen PT, Mullenax JG, Merigan TC. Inhibition of human cytomegalovirus replication by 9-(1,3-dihydroxy-2-propoxymethyl) guanine alone and in combination with human interferons. *Antimicrob Agents Chemother* 1984; **26**: 441-5.
- 21 Teich SA, Tay S, Friedman AH, Schmitterer ME. Viral particles in the conjunctiva of a patient with the acquired immune deficiency syndrome. *Am J Med* 1987; **82**: 151-2.

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