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A combination of low-dose chlorpromazine and neutralizing antibodies inhibits the spread of JC virus (JCV) in a tissue culture model: Implications for prophylactic and therapeutic treatment of progressive multifocal leukencephalopathy

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The human polyomavirus, JCV, is the etiologic agent of a fatal central nervous system (CNS) demyelinating disease known as progressive multifocal leukoencephalopathy (PML). PML occurs predominantly in immunosuppressed patients and remains an intractable complication in AIDS. To date, there are no effective therapies to treat PML. We previously demonstrated that the neuroleptic drug, chlorpromazine, inhibits the endocytic pathway used by JCV to infect glial cells. In this paper, we demonstrate that nontoxic doses of chlorpromazine are effective at inhibiting JCV multiplication and spread in a tissue culture model. The clinical efficacy of this drug or related compounds in treating PML has not been evaluated. *Journal of NeuroVirology* (2001) **7,** 307–310.

Keywords: central nervous system; drug therapy; JC virus; phenothiazines; progressive multifocal leukoencephalopathy

Chlorpromazine belongs to a class of drugs known as the phenothiazines. Its principal pharmacological effects are psychotropic. The drug also has sedative and antiemetic activity. It has been used clinically for the management of psychotic disorders, to control nausea and vomiting, for the relief of restlessness and apprehension before surgery, as an adjunct in the treatment of tetanus, and for relief of intractable hiccups. The major mechanism of action correlates with its ability to block dopaminergic receptors (Anden *et al*, 1970). Our interest in the drug was as an agent to selectively block clathrin-dependent endocytosis. At this level, chlorpromazine inhibits clathrin disassembly and receptor recycling to the plasma membrane (Hunt and Marshall-Carlson, 1986; Wang *et al*, 1993). We used this drug to demonstrate that infection of glial cells by JCV is mediated by clathrin-dependent endocytosis (Pho *et al*, 2000). In this study, we asked whether chlorpromazine could inhibit the spread of JCV in a tissue culture model system. We found that low doses of chlorpromazine together with neutralizing anti-JCV antibodies synergistically inhibited viral spread. At these lower doses, chlorpromazine by itself was not effective, and occasionally led to increased infectivity in the absence of neutralizing antisera. At higher doses, however, chlorpromazine inhibited infection without the addition of anti-JCV antisera. As the side effects of chlorpromazine include tardive dyskinesia and mild ataxia, its use in HIV-infected patients may not be warranted. Currently, we are testing other phenothiazine derivatives that have less toxic side effects for their ability to inhibit JCV infection of glial cells.

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Results

Low doses of chlorpromazine together with anti-JCV antisera inhibit viral spread in SVG-A cells Treatment of infected SVG-A cells with 100 ng/ml of chlorpromazine alone had no effect on viral spread

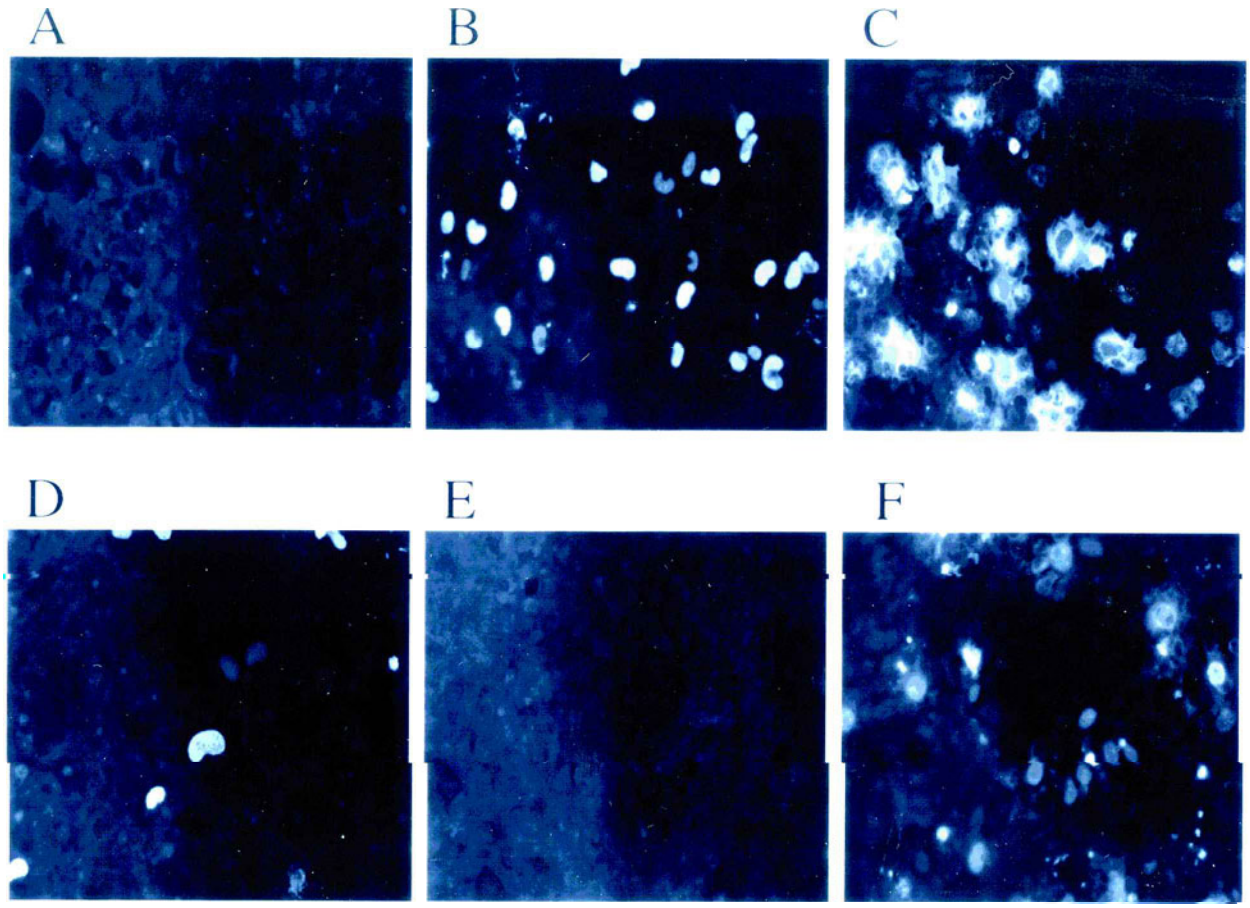

Figure 1 Low doses of chlorpromazine together with anti-JCV antisera inhibit viral spread in SVG-A cells. SVG-A cells were either uninfected (panel A) or infected with 205 HAU of JCV. At 24 hours postinfection the cells were not treated (panel B), treated with 100 ng/ml of chlorpromazine (panel C), treated with a 1:10,000 dilution of rabbit anti-JCV antisera (panel D), treated with chlorpromazine + anti-JCV antisera (panel E), or treated with chlorpromazine + preimmune antisera (panel F). At 1 week posttreatment, the cells were fixed and stained with an anti-VP1 monoclonal antibody followed by a goat anti-mouse FITC-labeled secondary antibody. The cells were washed and counterstained with Evan's blue. Magnification, 40X.

in SVG-A cells (Figure 1, panel C). Treatment of the cells with rabbit anti-JCV antisera reduced viral spread significantly when compared to treatment of the cells with preimmune sera (Figure 1, panels D and F). Chlorpromazine together with anti-JCV antisera cleared the infection (Figure 1, panel E).

Higher doses of chlorpromazine inhibit viral spread in the absence of anti-JCV antisera Infected cells were either untreated or treated with chlorpromazine at 10, 100, and 1000 ng/ml. Chlorpromazine at 10 ng/ml had no effect on viral spread (Figure 2). Chlorpromazine at 100 ng/ml increased the percentage of virus infected cells at 1 week postinfection (Figure 2). Chlorpromazine at 1.0 μg/ml significantly inhibited the percentage of virally infected cells. Chlorpromazine was toxic at 10 μg/ml (not shown).

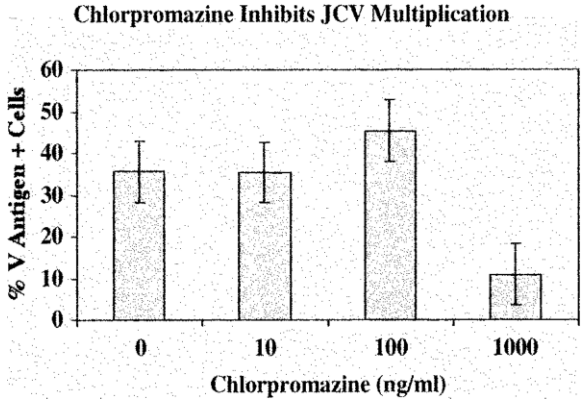

Figure 2 Higher doses of chlorpromazine inhibit JCV multiplication in SVG-A cells. SVG-A cells were infected with 205 HAU of JCV. At 24 h postinfection, the cells were untreated or treated with increasing doses of chlorpromazine as indicated. At 1 week postinfection, the percentage of infected cells was scored by indirect immunofluorescence assay using an anti-VP1 monoclonal antibody. Error bars represent the standard error of the mean between replicate samples.

Discussion

A number of anti-viral effects have been attributed to the neuroleptic drug chlorpromazine. It has

been shown to inhibit the uncoating of hepatitis A in BSC cells, to inhibit the multiplication of Junin virus, to inhibit SV40 DNA replication, and to inhibit gp 120-CD4 interactions and syncytia formation in T cells (Hirai *et al*, 1993; Candurra *et al*, 1996; Hewlett *et al*, 1997; Bishop, 1998). In this paper, we demonstrate that nontoxic doses of chlorpromazine effectively inhibit the multiplication of JCV in a tissue culture model. The mechanism by which chlorpromazine inhibits JCV multiplication and spread is not known but most likely involves an inhibition of clathrin-dependent endocytosis. The ability of chlorpromazine to inhibit JCV multiplication and spread was dose-dependent. At lower doses, chlorpromazine enhanced infection but this effect could be blocked by the inclusion of neutralizing anti-JCV antisera in the culture medium. It is unclear why lower doses of chlorpromazine would enhance infection.

One possibility is that partial inhibition of the endocytic pathway may lead to a compensatory upregulation of factors controlling endocytosis. The higher dose of drug may negate this effect. Unfortunately, the side effects of chlorpromazine include the induction of parkinsonian-like symptoms. This may be more pronounced in AIDS patients who already have basal ganglia deficits and the administration of chlorpromazine to these patients should be evaluated carefully. The administration of this drug to non-AIDS PML patients, however, may be warranted. We are currently screening other phenothiazine derivatives that have less toxic side effects for their ability to inhibit JCV multiplication. This is of crucial importance as there is currently no successful treatment for this uniformly fatal human demyelinating disease.

Materials and methods

Cells, virus, and antibodies The human glial cell line, SVG, was established by transformation of human fetal glial cells by an origin defective SV40 mutant and has been previously described (Major *et al*, 1985). The cell line was subcloned twice to generate SVG-A cells. SVG-A cells were maintained in a humidified 37°C CO_2 incubator in Eagles' Minimum Essential Media (E-MEM; Mediatech Inc, Herndon, VA) supplemented with 10% heat-inactivated fetal bovine serum (Mediatech, Inc, Herndon, VA). The hybridoma, PAB597, which produces an antibody to SV40 V antigen, was obtained from E. Harlow and maintained in RPMI-1640 Hybrimax® media (Sigma, St. Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (Mediatech Inc, Herndon, VA). The PAB597 monoclonal has previously been shown to cross-react with JCV VP1 (Atwood *et al*, 1995). Rabbit anti-JCV antisera was prepared by injecting a New Zealand White rabbit with purified JCV in incomplete Freund's adjuvant (Harlow and Lane, 1988). The rabbit was boosted twice with JCV incomplete Freund's adjuvant. The antisera was titered by ELISA and Western blot assays. Preimmune sera from this rabbit was used as a negative control. The hybrid Mad-1/SVEΔ virus was constructed by insertion of the regulatory region of SV40 into the regulatory region of the Mad-1 strain of JCV (Mad-1/SVE) (Vacante *et al*, 1989). Propagation of Mad-1/SVE in human glial cells led to deletions and alterations exclusively in the regulatory region. The rearranged regulatory region contains the origin of replication, the TATA box and 78 base pairs of the first 98-base pair repeat from JCV and one complete 72-base pair repeat from SV40. Most of one of the 72-base pair repeats and the 21-base pair repeats from SV40 were deleted. The virus is termed Mad-1/SVEΔ to indicate this fact. A comparison of the restriction patterns of Mad-1/SVEΔ DNA with the prototype Mad-1 DNA were identical except for the regulatory region changes just discussed (Vacante *et al*, 1989). No additional alterations were apparent following subsequent passage of Mad-1/SVEΔ in human fetal glial cells (Vacante *et al*, 1989). We sequenced the VP1 gene of the chimeric virus and it is identical to published sequence of VP1 from the prototype Mad-1 strain (Liu *et al*, 1998).

Inhibition of infection assay SVG-A cells growing on coverslips were incubated with 205 hemagglutination units (HAU) of JCV for 1 h at 37°C. The cells were then washed and incubated for an additional 24 h at 37°C so that the infection was well established. Infected cells were then incubated with increasing concentrations of chlorpromazine (1–1000 ng/ml) either alone or in the presence of a 1:10,000 dilution of either rabbit anti-JCV antisera or rabbit pre-immune sera. Controls included cells incubated with media alone, incubated with anti-JCV antisera alone, and incubated with pre-immune antisera alone. At 1 week postinfection, the cells were fixed in acetone and the percentage of infected cells scored by staining with an anti-V antigen monoclonal antibody.

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