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Clinical Trial Report

Convection-enhanced intraparenchymal delivery (CEID) of cytosine arabinoside (AraC) for the treatment of HIV-related progressive multifocal leukoencephalopathy (PML)

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AIDS-related PML continues to be a relatively common and rapidly fatal infection in patients with AIDS, and no effective therapy has been established to alleviate the effects of this disease. Through the years, isolated reports and small case studies have shown somewhat encouraging results using cytosine arabinoside (AraC) in the treatment of PML. The optimism behind the use AraC for this disease began to fade with ACTG trial 243, which suggested that AraC had no benefit in patients with HIV-related PML. In this article, we provide evidence that suggests that the failure of AraC in the ACTG trial may have been due to insufficient delivery of the drug through traditional intravenous and intrathecal routes. Furthermore, we provide evidence that convection-enhanced intraparenchymal delivery of AraC may prove to be a safe and effective means of treating this infection, and we outline a clinical trial that we have recently undertaken to test this hypothesis. *Journal of NeuroVirology* (2001) **7,** 382–385.

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Introduction

Progressive multifocal leukoencephalopathy (PML), resulting from JC virus infection of the brain, is a relatively common and rapidly fatal infection in patients with acquired immunodeficiency syndrome (AIDS). No effective therapy has been established to treat this infection, and it remains a major and growing concern for people with AIDS and their medical caregivers. The average survival after diagnosis of PML in HIV-infected subjects has been reported in different series from approximately 2.5 months to approximately 4 months, although some patients may have remission and prolonged survival or, rarely, spontanous recovery (Berger *et al*, 1997). To date, there is no clear method of predicting which patients are likely to have a more benign course.

A variety of different regimens have been used to treat PML with no clear evidence of efficacy. These include prednisone, acyclovir, adenosine arabinoside (both intravenously and intrathecally), and human-leukocyte-antigen-matched platelets. Through the years, isolated reports and small case studies have shown somewhat encouraging results using cytosine arabinoside (AraC). The largest reported experience is that of Britton (Britton *et al*, 1992). Thirteen of her AIDS patients with biopsy-proven PML were treated with intrathecal (IT) AraC after they had failed to improve or stabilize on maximally tolerated doses of ZDV and ddI. Eight of these patients stabilized, 4 patients up to 2 years and 4 patients up to 6 months. Although the prognosis was better in patients with a CD4 count of >200 cells/mm^3, most of those who improved on

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AraC had a CD4 count of <50 cells/mm^3. Clinical benefit was also reported using intravenous (IV) AraC in other studies (Portegies *et al*, 1991; Nicoli *et al*, 1992).

Because of the scattered reports about the efficacy of AraC in PML, the AIDS clinical trial group (ACTG) organized Trial 243, the objective of which was to compare treatment of PML in three arms: antiretroviral therapy (19 patients), antiretroviral therapy plus IV AraC (20 patients), and antiretroviral therapy plus IT AraC (19 patients). This trial was completed and reported in the *New England Journal of Medicine* (Hall *et al*, 1998). There was no difference in the survival of patients in any of the three arms, and the group concluded that AraC administered either IV or IT did not improve the prognosis of HIV-infected patients with PML. Somewhat remarkably, the authors of the ACTG Trial 243 did not address the reasons for the AraC failure. We believe the ACTG trial failed because of inadequate drug delivery.

Laboratory investigation

There is *in vitro* support for the concept that AraC is effective in impairing JC virus multiplication and replication. A preliminary form of this information was used by the ACTG trial to justify the use of AraC. Major and colleagues grew cell cultures from 16-week gestational age brain tissue and *in situ* hybridization and hemagglutination assays were used as a measure of viral DNA replication and multiplication, respectively. Of three drugs tested (AraC, ADC, and cidofovir) only AraC, at a concentration of 25 μg/ml, showed a significant effect in inhibiting JCV replication (Hou and Major, 1998). Major and coworkers have recently extended these studies. Persistently infected cells were treated with increasing concentrations of AraC for 3 weeks, harvested, and tested for JCV titers. At AraC concentrations of 40 μg/ml and greater, JC replication was completely inhibited. However, even at lower doses, there was a dose-effect relationship, with some effect at doses as low as 0.5 μg/ml. The relationship between these *in vitro* studies and the concentration of AraC needed to inhibit JC replication *in vivo* is unknown. Nevertheless, there is indeed evidence that AraC can suppress JC virus replication in a dose-dependent manner.

In the presence of a clearly demonstrable *in vitro* effect, we hypothesized that the lack of effectiveness of IV or IT AraC in the ACTG trial could be related to the failure of AraC to reach the target cells (Groothius *et al*, 1999). We conducted experiments to compare classical routes of delivery with convection-enhanced delivery (CED), which is a novel drug-delivery method in which the drug is infused directly into the brain under pressure (Bobo *et al*, 1994; Morrison *et al*, 1994; Laske *et al*, 1997; Groothius *et al*, 1999). Four groups of rats were administered 14C-AraC: 1) by bolus IV infusion, 2) by IT infusion into the cisterna magna, 3) by intraventricular (IVT) infusion into the lateral ventricle, and 4) by CED infusion into the caudate nucleus. To evaluate plasma and tissue AraC metabolism, distribution, and concentration, plasma and brain were evaluated with HPLC, and brain sections were analyzed by quantitative autoradiography. Although IV-administered AraC reached the entire brain, tissue levels were extremely low; the influx constant was 2.5 μl g^{-1} min^{-1}. This influx constant means that out of each ml of blood reaching the brain, only 0.00025% of the AraC reaches brain extracellular fluid. The brain efflux constant was 0.002 min^{-1}. Knowing the entrance rate and exit rate, the concentration of AraC in the brain extracellular fluid can be pharmokinetically modeled. After high-dose IV administration (3 g/m^2) of AraC, the mean maximum AraC plasma concentrations are 54.4 μM (Burk *et al*, 1997). Based on this value, maximum brain extracellular concentrations will be 5.4 μM, which is equivalent to 1.3 μg/ml. Thus, even in the setting of high-dose AraC IV administration, brain concentrations will be below the amount needed to suppress JC virus replication. After IT and IVT administration, tissue levels were high at the brain and ventricular surfaces and declined exponentially into brain tissue (Groothius and Levy, 1997; Groothius *et al*, 1999). These studies indicate that the IV, IVT, and IT routes of administration cannot deliver AraC in virally suppressive concentrations to brain.

In contrast to the inefficiency of IV and IT routes of delivery, 100% of drug delivered by CED reaches the brain extracellular space (Figure 1) and it is clearly possible to maintain AraC concentrations at those reported by Major (Hou and Major, 1998). Because the amount of AraC reaching brain extracellular fluid after CED is not a delivery issue, two additional aspects must be considered: neurotoxicity and the volume of brain reached by CED infusion. We have tested the neurotoxicity of AraC in mice and rats (Groothius *et al*, 1999). Concentrations of AraC from 10^{-3}–10^{-5} μM (0.0017–1,700 μg/ml) were infused into the caudate nucleus of rats and mice for 7 days. Neurotoxicity was evaluated with neuropathology and behavioral monitoring. Both measures indicated that extracellular AraC concentrations of <0.85 μg/ml (3.5 μM) were not neurotoxic, whereas concentrations >8.5 μg/ml (35 μM) were neurotoxic. In addition to the rat studies, we infused AraC into dogs. We infused AraC at a concentration of 25 μg/ml (100 μM) into a dog for 3 months at a rate of 3 μl/min without behavioral or pathological evidence of toxicity. An infusion concentration of 25 μg/ml is equivalent to an extracellular concentration of 125 μg/ml (500 μM). We are not sure whether the differences between the rat and dog were species-related, that the AraC was infused into gray matter in the rat versus into white matter in the dog, or due to the much larger volume of distribution in the dog. We believe that human infusions should

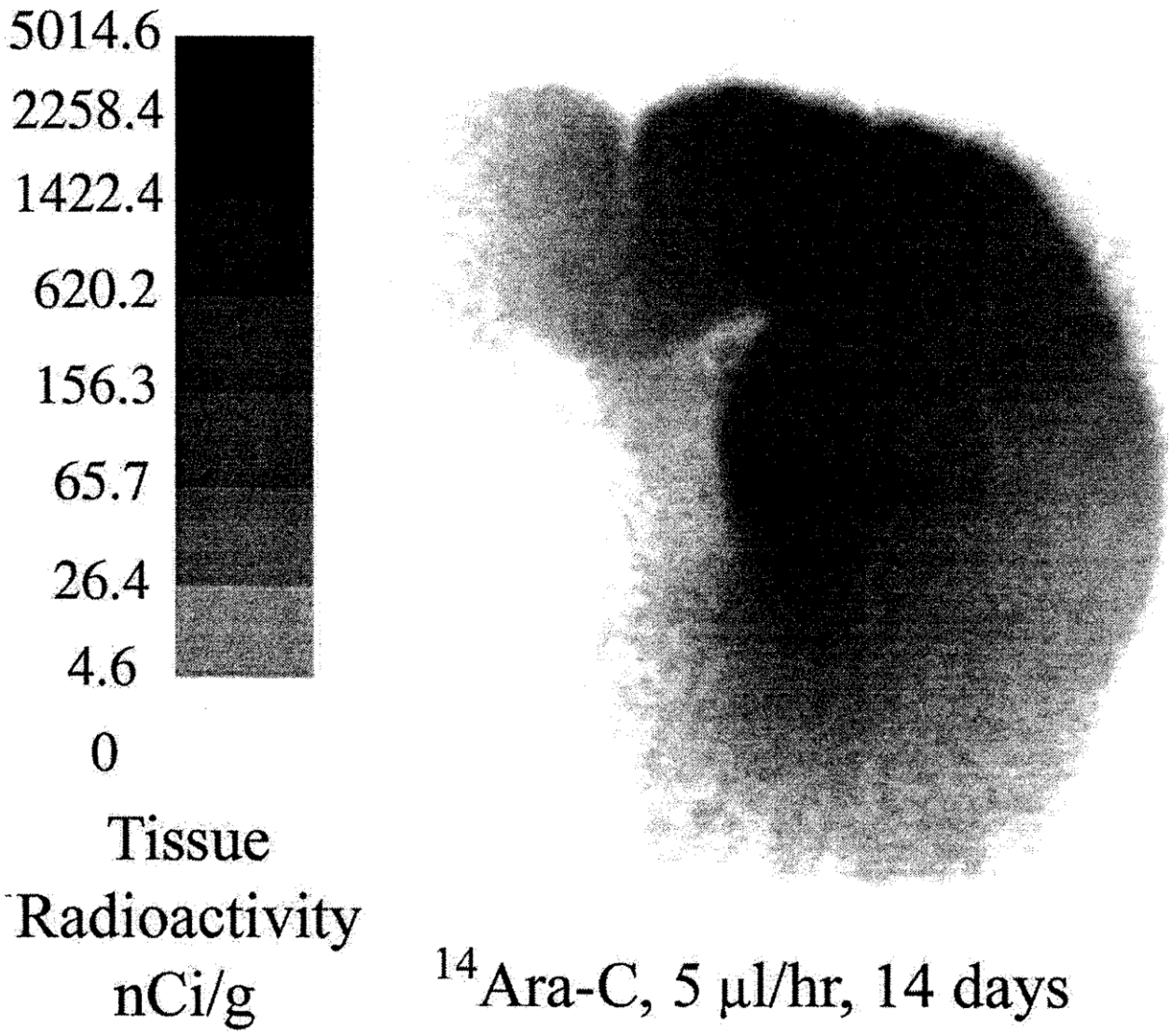

Figure 1 Rat coronal section at the level of the caudate nucleus, which was the target of this infusion. Note the high concentration of radiolabeled drug in the ipsilateral cortex.

resemble the dog, because in PML patients the drug will be infused into white matter, which permits much larger volumes of distribution. Our conclusion, based on these studies, is that CED can achieve virally suppressive tissue levels of AraC in the brain of animals without significant neurotoxicity.

The last issue that must be addressed before considering the use of CED of AraC to the brain is the volume of brain that can be reached. In mice and rats, the volume of brain reached by the infused drug is small. This has raised concern that the volume into which the drug distributes at effective concentrations, i.e., the volume of distribution (V_d), will be inadequate to reach brain cells infected by the JC virus. We have conducted extensive studies in dogs, which were done using CED of a water-soluble iodinated contrast agent to brain. In dogs, infusions were performed over a variety of infusion rates and durations, and the Vd was measured by using computed tomographic scanning. In these studies, the infusion reaches about 80% of the ipsilateral hemisphere with one infusion catheter. Contrast does not cross into the contralateral hemisphere or into the posterior fossa. We conclude from these studies that CED effectively delivers a water-soluble compound to one hemisphere in a dog model.

Convection-enhanced delivery of AraC in patients with HIV-1-related PML

Based on the evidence presented here, we believe that convection-enhanced delivery of AraC may be a safe and effective treatment for patients with HIV-1-related PML. As such, we have begun a nonrandomized, open-label, dose-escalating pilot

trial of continuous convection enhanced delivery of cytosine arabinoside into the brains of these patients. This study will recruit human subjects with HIV-related PML who have failed HAART therapy. At the time of initial stereotactic biopsy, subjects will have tissue obtained for histology, immunohistochemistry for PML, tissue for PCR studies and will have a specially designed intraparenchymal catheter inserted into the PML lesion. Sequentially, the subjects will receive an infusion of saline by CEID at a rate of 3 μl/min for 48 h, evaluation by MRI, and then infusion of AraC in a dose-escalation scheme beginning with an infusate concentration of 1 nM AraC, which will be increased every 2 days to 10 nM, 100 nM, 1 μM, 10 μM, and finally to 100 μM. Each subject will be evaluated by complete neurologic examination, CSF analysis, and MRI scanning every 2 days. If a subject tolerates the CEID infusion of 100 μM, AraC will be continued indefinitely. The goals of this trial are to document safety of CEID, safety of CEID infusion of AraC, and to collect preliminary data as to the efficacy of CEID infusion of AraC for the treatment of PML.

Conclusion

To date, patients with AIDS-related PML continue to have a poor prognosis as no effective treatment has been established for this infection. We believe that, based on *in vitro* and *in vivo* studies on animals, cytosine arabinoside (AraC) may prove to be a safe and effective drug for these patients. Furthermore, we believe that the apparent failure of AraC in ACTG Trial 243 to make a clinical difference in patients with AIDS-related PML was due to insufficient drug delivery to the infected lesions. It is our fervent hope that our clinical trial using CED of AraC will help to establish a safe and effective protocol to treat this deadly disease.

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