Biochemical Pharmacology, Vol. 32, No. 17, pp. 2455–2461, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

COMMENTARY

PROSPECTS FOR THE USE OF RADIOLABELED ANTIVIRAL DRUGS IN THE DIAGNOSIS OF HERPES SIMPLEX ENCEPHALITIS

RICHARD W. PRICE,*† YUTAKA SAITO* and JACK J. FOX‡

*Cotzias Laboratory of Neuro-Oncology and ‡Laboratory of Organic Chemistry, Memorial Sloan–Kettering Cancer Center, New York, NY 10021, U.S.A.

Herpes simplex virus (HSV) encephalitis is a sporadic disease accompanied by high mortality and severe morbidity in survivors [1–3]. With the recent advent of effective antiviral drugs, the disease can now be favorably influenced by therapy [2, 3], and the development of newer, more potent and selective antivirals offers the prospect of even greater efficacy. Whereas formerly the diagnosis of herpes simplex encephalitis was largely "academic", these therapeutic developments have fostered the need for accurate diagnosis [4]. Indeed, the inadequacies of current diagnostic methods may, even now, present the major impediment to effective management of the disease. When patients are treated early, at a stage when neurological deficit is minor, the outcome of herpes simplex encephalitis may be quite favorable, while delay in therapy until after the development of coma allows little improvement in outcome. Unfortunately, the clinical history and examination do not allow herpes simplex encephalitis to be clearly distinguished from a number of other encephalidites, and currently available non-invasive laboratory tests lack the precision necessary to establish a certain diagnosis [5, 6]. The CT scan may be normal early in the illness [5, 7], and characteristically the virus cannot be isolated from the cerebrospinal fluid [6]. Serologic techniques are not helpful early in the disease at a time when antiviral therapy should be instituted and even in retrospect, after convalescent specimens are examined, may not allow definitive analysis [6]. For these reasons, the only widely available, sufficiently accurate method of diagnosis involves brain biopsy and virus identification [5, 6]. However, brain biopsy is a controversial procedure which has not gained universal acceptance [8, 9]. Thus, at present the imprecision of non-invasive methods and the reluctance of clinicians to subject patients to brain biopsy may result in either delayed or inappropriate therapy of patients suffering, or suspected of suffering, from herpes simplex encephalitis. A clear need exists, therefore, for a rapid, sensitive and specific "non-invasive" method for the early diagnosis of this disease.

Strategy for the use of antivirals in diagnosis

To develop a new approach to the diagnosis of herpes simplex encephalitis, we have proposed to take advantage of the properties of recently developed antiviral drugs to develop an isotopically labeled probe which will allow regional brain infection to be identified *in vivo* using contemporary radionuclide scanning techniques [10]. In this approach, the antiviral drug provides diagnostic specificity while the imaging system permits anatomic resolution.

When productively infected by HSV, the host cell supports an ordered program of viral gene expression leading to the synthesis of viral progeny and eventual cell death [11]. The therapeutic efficacy of antiviral drugs depends on differences between this virus-directed metabolic program in the infected cell and host-determined metabolism of the uninfected cell. In the last several years a new generation of nucleoside analogues with potent anti-herpesvirus activity has been developed. Four of these drugs, acyclovir [9-(2-hydroxyethoxymethyl)guanine] [12], FMAU (2′-fluoro-5-methyl-1-β-D-arabinosyluracil) [13], FIAC (2′-fluoro-5-iodo-1-β-D-arabinosylcytosine) [14], and BVDU [*E*-5-(2-bromovinyl)-2′deoxyuridine] [15], are shown in Fig. 1. Acyclovir has been studied in the most detail and is the first of these to be approved for clinical use [16]. Its mechanism of action involves exploitation of two

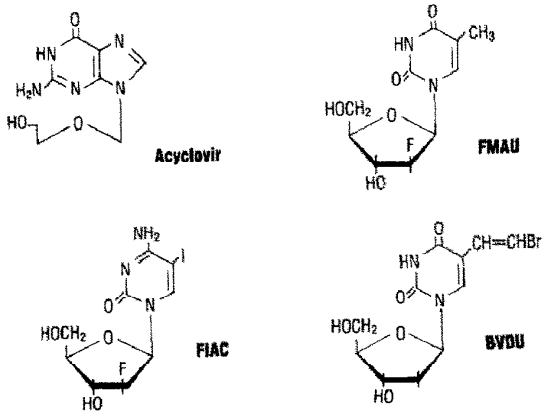

Fig. 1. Structures of four contemporary antiviral nucleosides.

† Send reprint requests to: Richard W. Price, M.D., Department of Neurology, Memorial Sloan–Kettering Cancer Center, 1275 York Ave., New York, NY 10021, U.S.A.

virus-coded enzymes, viral thymidine kinase (TK) and viral DNA polymerase [17–19]. As illustrated in Fig. 2, the nucleoside analogue is selectively phosphorylated to the mono-nucleotide by virus-coded TK (step 2 in the figure) and then converted to the nucleoside di- and triphosphates, probably by cellular kinases; the triphosphate, in turn, inhibits viral DNA polymerase (step 3 in figure). The other three drugs shown in Fig. 1 probably also achieve their antiviral activity by selective interaction with these two viral enzymes [13, 14, 20–22].

Our proposed use of this class of drugs for *in vivo* diagnosis relies principally on the first of these two stages of antiviral action, that involving viral TK. It is phosphorylation by this viral enzyme which accounts for the selective sequestration and concentration of these drugs within infected cells. Because the plasma membrane is relatively permeable to the nucleoside analogues, and in the absence of infection little or no phosphorylation occurs, these drugs readily diffuse into and out of the uninfected cell, as illustrated in Fig. 2 (step 1). In the HSV-infected cell, however, the drugs are phosphorylated by viral TK and, because the cell membrane is relatively impermeable to the resultant nucleotides, these metabolites are "trapped" intracellularly (Fig. 2, step 4). In this way, selective phosphorylation by viral TK establishes a gradient, allowing further drug entry and metabolism, resulting in concentration of the nucleotide derivatives (principally the nucleoside triphosphate) within infected cells. We reasoned that such trapping of isotopically labeled drug could be used to map infected cells *in vivo* and have reported recently the initial results of autoradiographic studies in an animal model which support the validity of this reasoning [10].

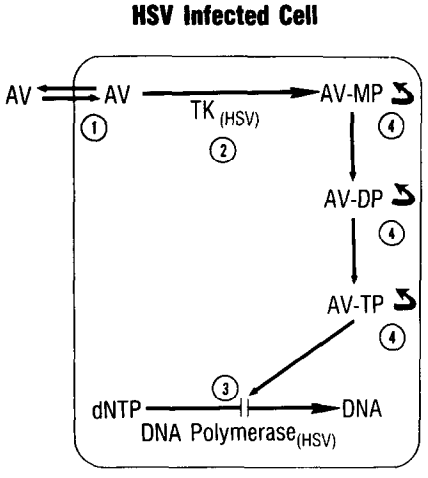

Fig. 2. Mechanism of antiviral activity and drug sequestration of infected cells by the current generation of anti-herpes agents. The antiviral nucleoside (AV): (1) crosses the cell plasma membrane, and (2) is then phosphorylated by virus-coded TK to the monophosphate nucleoside. (3) After additional phosphorylation, the triphosphate nucleoside then inhibits virus-coded DNA polymerase. (4) The nucleotide metabolites are unable to diffuse readily out of the infected cell, accounting for their sequestration.

Testing in vivo *uptake of a radiolabeled antiviral drug in an animal model using quantitative autoradiography*

We tested the strategy of using a labeled antiviral drug to detect regional infection in an animal model of focal HSV type 1 (HSV-1) encephalitis in conjunction with the technique of quantitative autoradiography ([10]; Y. Saito and R. W. Price, unpublished observations). A model of focal encephalitis in the rat was produced by intraocular HSV-1 inoculation in which infection follows neural pathways with major involvement of the optic pathway and its projections as well as several other neural structures with anatomic connections to the eye [23]. This model was chosen because it avoids direct brain injection with resultant tissue trauma and because a circumscribed focal encephalitis is produced. We used FMAU (Fig. 1) labeled with ^{14}C in the 2-position of the pyrimidine ring ([^{14}C]FMAU) (T-L. Su, K. A. Watanabe and J. J. Fox, to be published) for these initial studies. FMAU is a potent anti-herpes nucleoside which appears to parallel acyclovir in its mechanism of action [13]. Quantitative autoradiographic methods were used to evaluate regional tissue drug distribution in a similar manner to those developed by Sokoloff and colleagues for evaluation of regional cerebral glucose metabolism with [^{14}C]2-deoxyglucose [24, 25]. In brief, at various intervals after intravenous [^{14}C]FMAU injection, infected animals were killed, and their brains were quickly removed and frozen. Serial cryostat-cut 20 μm brain sections were then used to prepare autoradiograms on X-ray film. In selected animals, sections were also prepared from a variety of visceral organs. Using a series of ^{14}C-standards for calibration, regional brain concentrations of isotope were then calculated using a computerized image-processing system. The distribution of labeled drug was correlated subsequently with that of viral antigen as defined by peroxidase-anti-peroxidase immunological staining of adjacent tissue sections. A high correlation between increased ^{14}C-activity and viral antigen was found, allowing infected areas to be readily discriminated from background uninfected brain (Fig. 3). Ratios of activity in infected compared to uninfected regions were found to depend on the density of infected cells and the interval between isotope administration and animal sacrifice. For example, in animals killed 6 hr after drug injection, ratios of ^{14}C-activity in infected compared to uninfected brain ranged from between 2 to 1 and 15 to 1. When infected rats were killed at later intervals (for example, at 24 or 48 hr after isotope injection), background brain radioactivity was reduced to negligible levels while isotope persisted within infected regions.

Two exceptions to the clear distinction between infected and uninfected nervous system structures were noted. The first of these involved the choroid plexus. Although FMAU enters the brain, a permeability "barrier" slows this entry such that a delay of 30–90 min intervenes before peak brain concentrations are reached and even then the concentration of FMAU in uninfected brain remains below that of plasma ([10]; F. S. Philips, A. Feinberg, T-C. Chou, P. M. Vidal, T-L. Su, K. A. Watanabe and J. J. Fox, unpublished). On the other hand, because, as

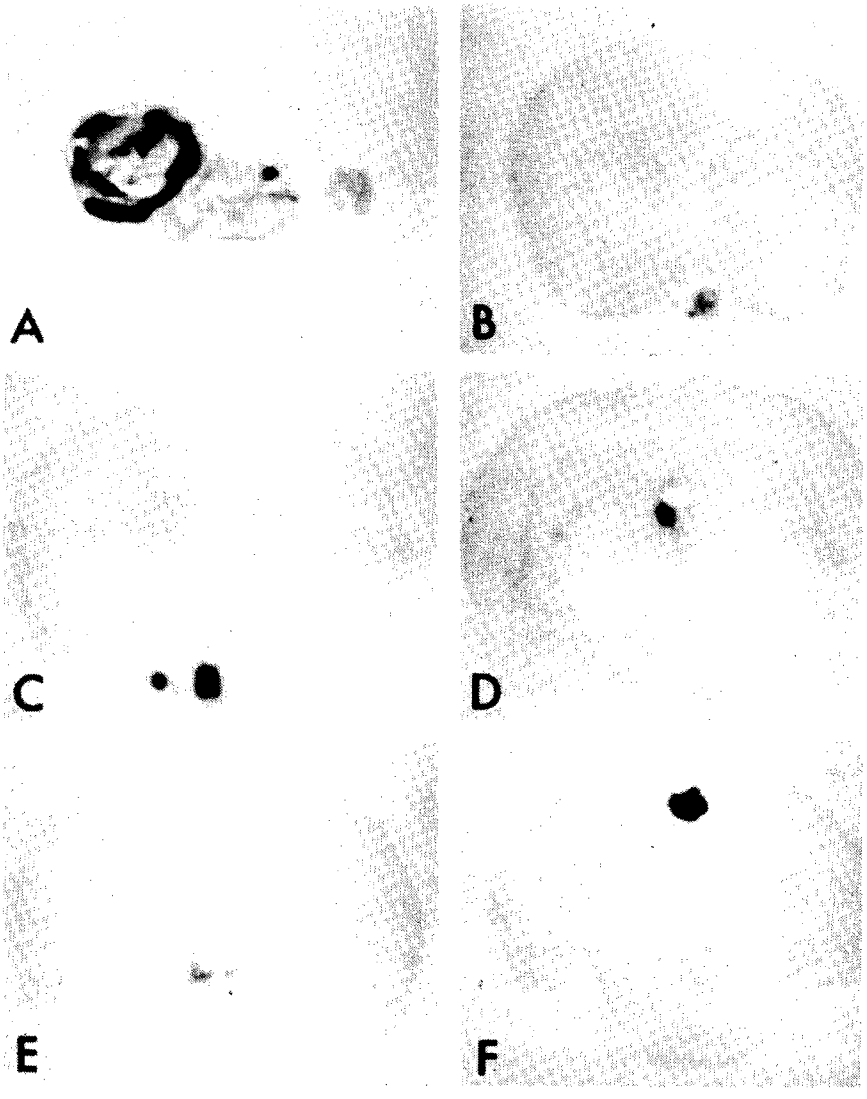

Fig. 3. Focal uptake of [^{14}C]FMAU in HSV-1 infected regions of rat brain. Shown are computer reconstructions of autoradiograms prepared from sections of an HSV-1 infected rat killed 6 days after intraocular viral inoculation and 6 hr after intravenous [^{14}C]FMAU injection. (A) The high drug concentration in the infected retina and cornea contrast with adjacent non-infected tissue including the extraocular muscles in this sagittal section of the inoculated eye. (B) The infected optic nerve can be distinguished inferiorly by enhanced isotope uptake, and uptake can also be detected in the non-infected periventricular regions in this coronal brain section. (C) Marked [^{14}C]FMAU uptake is present in the infected hypothalamus bilaterally (principally in the suprachiasmatic nuclei) and in the left optic tract (seen on the left side of the image). (D) An area of intense drug uptake associated with infection of the dorsal medial thalamus is present. (E) Less intense uptake of drug in the central mesencephalon associated with infection is present. (F) Very high [^{14}C]FMAU uptake is noted in the infected pineal gland.

in most visceral organs, no barrier retards rapid drug diffusion into the choroid plexus, concentrations in the latter closely paralleled those in plasma. Because of this discrepancy in permeability, [^{14}C]FMAU activity in the choroid plexus exceeded that of uninfected brain, particularly early after drug administration. Thus, at 2 hr after injection choroid plexus activity was 3–4 times that of brain and even at 6 hr was 1.5 to 2.5 times as great. This resulted in overlap of drug concentrations in the choroid plexus and infected brain regions, and points out that extension of the proposed strategy to human diagnosis must take into account differences in kinetics of drug distribution between normal brain parenchyma and adjacent tissues with different permeability characteristics such as the choroid plexus.

The second exception involved the surface of the lateral ventricles, where a thin layer of increased concentration of labeled drug was noted, probably within, or adjacent to, the ependymal lining (Fig. 3). The mechanism of drug concentration in this area differed from that in choroid plexus. Enhanced radioactivity in this periventricular zone was not appreciated early after isotope injection, but rather

was only noted when activity persisted in this thin layer of cells as the drug concentration declined in the uninfected brain parenchyma. In fact, activity remained in this zone for 48–96 hr after injection, which were the latest intervals tested, despite virtually complete clearing of drug from the remainder of the normal brain. We interpret this finding as likely due to "nonselective" uptake and metabolism of drug related to continued cellular DNA synthesis in cells of this region in young rats [26]. This is supported by the observation that the kinetics of [^{14}C]FMAU appearance and persistence in this periventricular zone closely paralleled that observed in rapidly dividing cell populations of certain visceral organs, including the spleen and intestine (Y. Saito and R. W. Price, unpublished). In this regard, our quantitative autoradiographic studies revealed a degree of nonselectivity of [^{14}C]FMAU uptake presumably related to phosphorylation by cellular TK in these dividing cells. This represents an important potential shortcoming of FMAU for human diagnostic application.

Despite these exceptions, our initial studies provide a foundation for optimism that the proposed diagnostic strategy can be applied to humans. They revealed regional drug concentrations sufficient to allow imaging of focal infection in contrast to noninfected brain tissue. The studies also demonstrated the value of quantitative autoradiography as a method for establishing an experimental foundation upon which to base consideration of human application. Simple scintillation counting of brain homogenates or biochemical analysis of nucleotides would likely have failed to detect small areas of high drug concentration related to infection because of dilution by the large volume of uninfected brain. In addition, these other techniques do not allow easy correlation with regional infection by parallel immunological staining. It is likely that a previous study of labeled acyclovir in infected brain failed to detect enhanced drug concentration simply because of the insensitivity of the techniques used [27]. In addition, quantitative autoradiography, in revealing enhanced activity in the choroid plexus and certain rapidly dividing cell poplulations, has defined two classes of potential false-positive imaging which must be taken into account when extending this strategy to humans: (1) differences in isotope concentrations related to variable drug delivery and permeability of tissues, and (2) differences related to metabolism and uptake of the drug by noninfected cells. The implications of these observations for human application will be considered below.

Human HSV encephalitis

Before considering application of the outlined strategy to man, it is useful to consider some of the features of human herpes simplex encephalitis that may bear on its implementation. Of particular importance are the anatomic distribution of infection, the influence of the survival time of infected cells, and the epidemiology of the disease. There are, in fact, two types of herpes encephalitis, an adult type caused by HSV-1 and a neonatal encephalitis resulting principally from HSV type 2 infection. In the foregoing and following discussion, we consider only the adult type and use the term herpes simplex encephalitis as synonymous with that disease. Neonatal infection differs in many respects [28], and will not be discussed further. However, similar principles could also be applied to the use of radiolabeled antivirals in that disease as well. The adult type of encephalitis, which may afflict adults or children, is an illness in which a number of fundamental pathogenetic questions remain unanswered [29, 30]. Patients who are otherwise well, some with and some without a history of previous benign HSV infections (e.g. cold sores), are stricken with a devastating encephalitis in the absence of a defined provocative cause. One of the remarkable features of the illness is its topography. Characteristically, the medial and inferior temporal lobe, the inferior frontal lobe, the cingulate gyrus, insula, and certain other "limbic" structures, either unilaterally or more commonly bilaterally, are predominantly affected [31]. This localization has raised speculation that the brain may be seeded via olfactory pathways. Whatever the reason, this distribution accounts for a certain stereotypy in the clinical presentation of patients. The fact that the infection is focal with a predilection for these particular structures may bear on the success of clinical imaging using an antiviral radiopharmaceutical. Thus, the imaging technique must be adequate to detect a circumscribed, presumably small, focus of infected cells present early in the course of the disease and to distinguish this focus both from adjacent non-parenchymal normal structures, such as the choroid plexus of the temporal horn and blood vessels at the base of the brain, as well as from other pathological conditions accompanied by either altered permeability or potential nonselective drug metabolism. As discussed below, high specificity of the antiviral probe and high resolution of the imaging technique may be required to overcome these potential difficulties.

The lifespan of infected cells *in vivo* may influence the capacity of antiviral radiopharmaceuticals to image brain infection. Once a cell is infected by HSV, there is a time delay until viral TK is expressed and a further delay until maximal drug-concentrating capacity within the infected cell is achieved. It may be speculated that the immune defenses of afflicted individuals will influence the power of the proposed technique in detecting infection if immune-mediated cell lysis reduces the survival time of infected cells and thereby truncates the period of potential active drug uptake. However, because TK expression precedes viral DNA synthesis and replication of progeny, actively spreading infection should be detected. In addition, recent studies by Esiri of viral antigen in the brains of autopsied cases of HSV encephalitis suggest that, at least in severely afflicted patients, immune lysis may not be rapid, but rather that a considerable lag may exist between spread of infection and the subsequent inflammatory reaction [31]. In Esiri's material, many areas of infection exhibited minimal tissue reaction and resembled the appearance in our rat model of encephalitis when immunosuppression was induced with cyclophosphamide [10, 23]. Such areas of infection without inflammation were readily mapped by [^{14}C]FMAU autoradiography in the rat brain, indicating that

severe tissue necrosis and marked disruption of the blood–brain barrier was not required for such imaging, and that delay in immune lysis might even facilitate detection by prolonging the period of drug uptake.

An additional factor which will influence the eventual implementation of the proposed diagnostic methodology is the epidemiologic setting of HSV-1 encephalitis. It is an uncommon and sporadic disease. This uncommon occurrence may not only diminish its consideration in the differential diagnosis by clinicians but, more pertinently, must also be taken into account when planning clinical application of this strategy. If, for example, only one or two patients in which the disease is a major consideration are admitted to a given hospital yearly, it may be difficult to maintain the ready availability of the technology (particularly the isotope-labeled drug) for the requisite rapid diagnostic procedure needed to establish an early diagnosis. On the other hand, widespread availability of a safe and specific test may foster more frequent diagnostic consideration of HSV infection, and the capacity to detect circumscribed infection may even lead to definition of heretofore unrecognized, more benign forms of the disease, further expanding the indications for the test.

Extension to human disease

In extending the use of antiviral radiopharmaceuticals to human diagnosis, three interdependent aspects require consideration: the drug to be used as a probe, the isotope with which it is labeled, and the imaging system (Table 1). Each of these will bear on the sensitivity to detect a small focus of infection and the capacity to discriminate such infection from both normal tissues and other pathological conditions.

Table 1. Factors affecting sucess of selective imaging of infected brain regions

A.	Drug
	1. Slectivity of metabolism by cells expressing viral TK
	2. Amenability to convenient isotope labeling
	3. Biodistribution
	a. Kinetics of systemic distribution
	b. Systemic metabolism
	i. Phosphorylation with intracellular trapping
	ii. Fate of isotope label
	c. Brain permeability
B.	Isotope
	1. Energy spectrum
	a. Imaging
	b. Toxicity
	2. Half-life
	a. Convenience of shelf-life
	b. Toxicity
C.	Imaging system
	1. Anatomic resolution
	a. Two-dimensional
	i. Standard gamma scanning
	b. Three-dimensional
	i. Single-photon emission tomography
	ii. Positron emission tomography
	2. Isotope requirements
	a. Gamma emission
	b. Positron emission

The probe. Development of a suitable radiolabeled antiviral drug probe must take into account the specificity of metabolism of the drug by virus-infected cells, the capability and convenience of labeling the drug without altering that specificity, and the biodistribution of the drug.

Ideally, the drug to be used should be phosphorylated exclusively by viral TK and, therefore, label only infected cells. Deviation from this ideal may result in loss of diagnostic precision and enhanced radiation toxicity, depending on other conditions of the test system. Loss of specificity was noted in our [^{14}C]FMAU studies in which the drug was trapped in the periventricular zone. This is unlikely to present a problem in humans, but other DNA-synthesizing cells in the brain, including neoplastic, inflammatory, or even reactive glial cells, might also take up the radiopharmaceutical if it can serve as substrate for cellular kinases. Similarly, drug sequestration within proliferating cells in the viscera may enhance the radiation toxicity to a particularly vulnerable population (depending on the isotope dosimetry and half-life). Hopefully, one or more of the other nucleoside analogues currently under investigation will prove superior to FMAU in this regard. If not, testing of additional analogues may be warranted. Because inhibition of viral replication is most likely a two-step process involving interaction with both viral TK and DNA polymerase, as discussed above, and because diagnostic capability requires only the first of these steps, antiviral potency may not fully predict differential sequestration of drug by infected cells. Thus, in theory, a compound may be selectively phosphorylated by viral TK but the resultant nucleoside triphosphate may only weakly inhibit viral DNA synthesis. Screening for a diagnostic probe would thus best directly test drug uptake by infected cells. On the other hand, with presently available drugs, a minor degree of uptake by noninfected dividing cells in visceral organs and even within the brain may be tolerable, depending on the character and dosage of the isotope label and the resolution of the imaging system to be used.

In addition to its selective sequestration by HSV-infected cells, in order to serve as a diagnostic probe the antiviral drug must be amenable to isotopic labeling. Because the antiviral activity related to differential phosphorylation of this class of compounds by viral TK varies considerably with minor molecular manipulations [13, 32, 33], coupling the drug with an exogenous radioactive "tag" is likely to alter this specificity, and isotope substitution of a molecule intrinsic to the structure of the drug is probably required. If this proves to be the case, then the structure of the drug must be amenable to such substitution with a useful isotope. In this regard, acyclovir (Fig. 1) may provide little opportunity for labeling unless ^{11}C is used for positron emission tomography. For this reason our current efforts are directed at substituting a radiolabeled iodine for that in FIAC (Fig. 1) in order to develop a radiopharmaceutical suitable for gamma detection. A second strategy under consideration is substitution of either fluorine with ^{18}F or a carbon with ^{11}C in FIAC for use in positron emission tomographic studies. To develop practical methods of producing clinically

useful radiopharmaceuticals, new synthetic pathways may need to be developed in which the isotope is added at the terminal step or is substituted for an unlabeled molecule after completed synthesis.

The kinetics of biodistribution and metabolism of the antiviral drugs bear on their use as probes in a number of aspects, including those related to successful imaging and to potential toxicity. Discrimination of an infected focus depends on the contrast of isotope in the focus to that in background tissues. The first requirement is that the drug must reach the infected cell so that selective trapping can take place. Although a permeability barrier slows inward diffusion, these nucleoside analogues cross into the brain whether or not infection is present. However, reduced brain permeability underlies the differential concentration of isotope in brain compared to choroid plexus seen during the earlier intervals after [^{14}C]FMAU administration in our experimental studies. Such differences in tissue permeability must be taken into account when planning and interpreting human studies. Thus, higher drug concentrations may be seen in the choroid plexus, extraneural structures, and even within the brain if the permeability differs from that of normal brain (as it might in a tumor or other pathological condition where permeability and drug delivery confer tissue characteristics similar to those of the choroid plexus or systemic organs such as the liver). Of particular importance will be the timing of imaging after drug administration. A longer interval between drug administration and scanning will favor detection of differential drug sequestration rather than simply drug entry. As further experimental data become available, more rigorous modeling of drug kinetics in both uninfected and infected tissues can be carried out to help define optimal conditions for imaging human infection. Systemic drug distribution and metabolism will also define potential radiation toxicity to particular organs in relation not only to the parent compound but also to metabolites. For example, if an isotope of iodine is used to label FIAC, it will be important to assess release of free iodine such that subsequent uptake by the thyroid can be blocked with "cold" iodide.

Isotope. The isotope to be incorporated into the diagnostic probe will be constrained on the one hand by the structure of the antiviral drug and on the other by the imaging system to be employed. As discussed above, the radionuclide most likely must be substituted into the basic structure of the antiviral nucleoside analogue without altering its property as a substrate for viral TK. In addition, the imaging techniques to be considered below possess different isotope requirements. Isotopes such as ^{18}F with very short half-lives have an advantage in reducing prolonged radiation exposure in the event that the probe is "non-specifically" sequestered by rapidly dividing cells, while longer-lived isotopes, by virtue of their longer shelf-life, eliminate the necessity of resynthesis on very short notice. Also, if pathogenetic studies of drug sequestration over longer periods are considered, they will require longer-lived isotopes so that scanning can be done at late intervals after probe administration.

Imaging system. Three contemporary methods of imaging can be considered for clinical use in detecting a radiolabeled antiviral probe: standard gamma scanning, single-photon emission tomography and positron emission tomography. For each of these, arguments pro and con can be advanced. Standard gamma camera scanning holds the major advantage of ready applicability using equipment now widely available. If an isotope probe suitable for standard techniques is made available, information regarding the usefulness of this approach to diagnosis and its implementation could appear rapidly. Isotopes with longer half-lives would allow practical commercial development of a suitable probe with a shelf-life adequate for storage and shipping. A major problem with this technique is likely to result from its limited spatial resolution. Because, as discussed earlier, HSV replication is concentrated in portions of the brain adjacent to the base of the skull where drug penetration into non-neural structures may exceed that of normal brain, early focal infection may not be resolved by two-dimensional scanning.

A method of three-dimensional image reconstruction may therefore be needed to clearly detect early focal HSV-1 infection. Single-photon emission tomography [34] in this regard may be more suitable for use with a gamma-emitting radionuclide such as ^{123}I. This technique is superior to conventional gamma scanning in that collimation of emitted energy and computerized reconstruction allow higher resolution and clearer definition of structures deep in the brain. This is a technique which is gaining wider usage and is likely to be more readily available than positron scanning in the next several years. An additional advantage of this technique over positron emission tomography is the relative stability of the isotopes used which do not require an in-house radiopharmaceutical production unit.

On the other hand, of available techniques positron emission tomographic scanning [35] is likely to offer the greatest resolution and therefore superior diagnostic capability, particularly with respect to smaller foci of infection. Positron emission tomography may therefore also be more suitable for pathogenetic studies aimed at tracing the progress of infection in the brain and, perhaps, in the peripheral nervous system as well. The major disadvantages of positron scanning include limited availability to a few research centers, need for an extensive support team, and the short-lived nature of the isotopes.

An additional imaging technique which possibly could be exploited in analogous fashion in the more distant future is that of nuclear magnetic resonance (NMR). This technology is currently evolving rapidly and shows enormous potential for non-invasive tissue imaging [36]. For specific identification of HSV infection, technological advances would need to allow detection and spatial resolution of an isotopically altered (e.g. ^{13}C) antiviral. The advantage of this approach would include the stability and safety of the probe as well as relief from the restriction of confining the basic drug species to those containing molecules which can be substituted with a radioactive isotope (e.g. those containing iodine, fluorine or bromine).

Conclusions

The use of isotopically-labeled antiviral drugs as probes for the diagnosis of herpes simplex encephalitis holds considerable promise. Proper implementation of the outlined strategy should allow early specific diagnosis of HSV encephalitis, hopefully supplanting brain biopsy. In addition, the technique may be of value in studying the pathogenesis of HSV infections as well as in following the course of therapy. As enumerated above, a number of impediments are yet to be overcome before this strategy is applied to man. Further consideration to isotopic labeling of currently available drugs or exploration of the use of additional related compounds in order to develop specific and convenient probes for either gamma or positron scanning will be needed. As demonstrated by our preliminary efforts, the methodology of quantitative autoradiography should continue to prove valuable in studies of animal models. With additional data describing systemic biodistribution, brain permeability, and metabolism of these drugs in the presence and absence of infection, more rigorous modeling can be carried out to guide optimal dosimetry and timing for the various scanning methods. Practical synthetic methods for preparation of the labeled probe will also need to be developed. Overall, the obstacles to advancing this diagnostic strategy appear amenable to solution and should allow assessment in man in the not-too-distant future. It is hoped that enhanced diagnosis and clinical management of herpes simplex encephalitis and related viral infections will result from such an effort.

Acknowledgements—This work was supported by the Memorial Sloan–Kettering Cancer Center Neurology Service Fund and U.S. Public Health Service Research Grants NS 12396 and CA 18601. R.W.P. is an Irma T. Hirshl Career Scientist Awardee, and Y.S. is a Visiting Fellow supported by the Tokyo Medical College. We thank our colleagues David A. Rottenberg, Jerome B. Posner, Gavril W. Pasternak, and Steven Lukes for their helpful suggestions and Adele Ahronheim for preparation of the manuscript.

REFERENCES

1. J. R. Baringer, in *Handbook of Clinical Neurology* (Eds. P. J. Vinken and G. W. Bruyn), Vol. 34, p. 145. North-Holland, Amsterdam/New York (1978).
2. R. J. Whitley, S-J. Soong, R. Dolin, G. J. Galasso, L. T. Ch'ien, C. A. Alford and the NIAID Collaborative Study Group, *New Engl. J. Med.* **297**, 289 (1977).
3. R. J. Whitley, S-J. Soong, M. S. Hirsch, A. W. Karchmer, R. Dolin, G. Galasso, J. K. Dunnick, C. A. Alford and the NIAID Collaborative Antiviral Study Group, *New Engl. J. Med.* **304**, 313 (1981).
4. R. J. Whitley and C. A. Alford, *J. Am. med. Ass.* **244**, 371 (1980).
5. R. J. Whitley, S-J. Soong, C. Linneman, Jr., C. Liu, G. Pazin, C. A. Alford and the NIAID Collaborative Antiviral Study Group, *J. Am. med. Ass.* **247**, 317 (1982).
6. A. J. Nahmias, R. J. Whitley, A. N. Visintine, Y. Takei, C. A. Alford and the NIAID Collaborative Antiviral Study Group, *J. infect. Dis.* **145**, 829 (1982).
7. S. B. Greenberg, L. Taber, E. Septimus, S. Kohl, J. Puck and R. N. Bryan, *Archs Neurol., Chicago* **38**, 58 (1981).
8. M. Barza and S. G. Pauker, *Ann. intern. Med.* **92**, 641 (1980).
9. P. Braun, *Am. J. Med.* **69**, 895 (1980).
10. Y. Saito, R. W. Price, D. A. Rottenberg, J. J. Fox, T-L. Su, K. A. Watanabe and F. S. Philips, *Science* **217**, 1151 (1982).
11. P. G. Spear and B. Roizman, in *DNA Tumor Viruses. Molecular Biology of Tumor Viruses* (Ed. J. Tooze), 2nd Edn, Part 2, p. 615. Cold Spring Harbor Laboratory, New York (1980).
12. G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp and H. J. Schaeffer, *Proc. natn. Acad. Sci. U.S.A.* **74**, 5716 (1977).
13. J. J. Fox, C. Lopez and K. A. Watanabe, in *Medicinal Chemistry Advances* (Eds. F. G. de las Heras and S. Vega), p. 27. Pergamon Press, Oxford (1981).
14. C. Lopez, K. A. Watanabe and J. J. Fox, *Antimicrob. Agents Chemother.* **17**, 803 (1980).
15. E. De Clercq, J. Descamps, P. De Somer, P. J. Barr, A. S. Jones and R. T. Walker, *Proc. natn. Acad. Sci. U.S.A.* **76**, 2947 (1979).
16. D. H. King and G. Galasso (Eds.), *Proceedings of a Symposium on Acyclovir sponsored by Burroughs Wellcome Co. and the National Institute of Allergy and Infectious Diseases.* American Journal of Medicine, Vol. 73 Supplement, New York (1982).
17. J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller and G. B. Elion, *J. biol. Chem.* **253**, 8721 (1978).
18. D. M. Coen and P. A. Schaffer, *Proc. natn. Acad. Sci. U.S.A.* **77**, 2265 (1980).
19. L. E. Schnipper and C. S. Crumpacker, *Proc. natn. Acad. Sci. U.S.A.* **77**, 2270 (1980).
20. H. S. Allaudeen, J. Descamps, R. K. Sehgal and J. J. Fox, *J. biol. Chem.* **257**, 11879 (1982).
21. H. S. Allaudeen, M. S. Chen, J. J. Lee, E. De Clercq and W. H. Prusoff, *J. biol. Chem.* **257**, 603 (1982).
22. C. S. Crumpacker, L. E. Schnipper, P. N. Kowalsky and D. M. Sherman, *J. infect. Dis.* **146**, 167 (1982).
23. Y. Saito and R. W. Price, *Trans. Am. neurol. Ass.* **106**, 284 (1981).
24. L. Sokoloff, M. Revich, C. Kennedy, M. H. Des Rosiers, C. S. Patlak, K. D. Pettigrew, O. Sakurada and M. Shinohara, *J. Neurochem.* **28**, 897 (1977).
25. L. Sokoloff, *J. cereb. Blood Flow Metab.* **1**, 7 (1981).
26. B. Schultze and H. Korr, *Cell Tissue Kinet.* **14**, 309 (1981).
27. K. K. Biron, J. E. Noblin, P. de Miranda and G. B. Elion, *Antimicrob. Agents Chemother.* **21**, 44 (1982).
28. R. J. Whitley, A. J. Nahmias, A. M. Visintine, C. L. Fleming, C. A. Alford and the NIAID Collaborative Antiviral Study Group, *Pediatrics* **66**, 489 (1980).
29. R. J. Whitley, A. D. Lakeman, A. Nahmias and B. Roizman, *New Engl. J. Med.* **307**, 1060 (1982).
30. L. E. Davis and R. T. Johnson, *Ann. Neurol.* **5**, 2 (1979).
31. M. M. Esiri, *J. neurol. Sci.* **54**, 209 (1982).
32. Y-C. Cheng, G. Dutschman, J. J. Fox, K. A. Watanabe and H. Machida, *Antimicrob. Agents Chemother.* **20**, 420 (1981).
33. E. De Clercq, J. Descamps, G. Verhelst, R. T. Walker, A. S. Jones, P. F. Torrence and D. Shugar, *J. infect. Dis.* **141**, 563 (1980).
34. P. J. Ell, O. Kahn, P. H. Jarritt and I. D. Cullum, *Radionuclide Section Scanning—An Atlas of Clinical Practice.* Grune & Stratton, New York (1982).
35. M. M. Ter-Pogossian, M. E. Raichle and B. E. Sobel, *Scient. Am.* **243**, 171 (October 1980).
36. L. Kaufman, L. E. Crooks and A. R. Margulis (Eds.), *Nuclear Magnetic Resonance Imaging in Medicine.* Igaku-Shoin, Tokyo (1981).