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Trifluorothymidine: potential non-invasive diagnosis of herpes simplex infection using $^{19}$F nuclear magnetic resonance in a murine hepatitis model

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(Accepted 8 September 1987)

Summary

Trifluorothymidine (TFT) is known to be concentrated in herpes simplex virus (HSV) infected cells in vitro in the form of phosphorylated derivatives. We studied a murine hepatitis model of HSV infection to determine whether this in vitro observation would also be demonstrable in vivo. Following i.v. injection of 100 or 160 mg/kg TFT, TFT was found in significantly higher concentrations in the livers of HSV-2 infected mice than in the livers of uninfected mice, mice infected with murine hepatitis virus (MHV-A59) or mice with hepatitis from carbon tetrachloride treatment. Neither altered renal function, nor altered pharmacokinetics could account for this difference. $^{19}$F Nuclear Magnetic Resonance spectroscopy readily detected the $^{19}$F from TFT in both liver extracts and whole livers, particularly at higher tissue levels, i.e. $> 50 \mu$g/g tissue. If further studies with living animals support these preliminary observations, clinical application could be pursued.

Trifluorothymidine; Herpes simplex virus; Herpes simplex hepatitis; $^{19}$F Nuclear magnetic resonance; Carbon tetrachloride hepatitis

Supported in part by a grant from the Veterans Administration, and by grant 1 RO 1 EY05800 from the National Institutes of Health.

Presented in part at the 87th meeting of the American Society for Microbiology, Atlanta, GA. March, 1987.

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Introduction

A number of antiviral drugs exhibit marked accumulation in herpes simplex virus (HSV) infected cells in vitro. The virus induced enzyme thymidine kinase (TK) phosphorylates these drugs to the monophosphate, which is converted to the di- and the triphosphate by cellular enzymes. Such a mechanism has been shown to lead to the intracellular accumulation of phosphorylated derivatives of acyclovir (Furman, 1981), 9-(2-hydroxyethoxymethyl)guanine (DHPG) (Smee, 1985), trifluorothymidine (TFT) (Fischer, 1983) and other agents (Price, 1986). TFT contains 3 fluorine atoms which comprise approximately 19% of its molecular weight. Since the naturally occurring isotope, $^{19}\text{F}$, has a nuclear spin of 1/2, it is possible that sufficient concentrations of TFT could be detected by nuclear magnetic resonance (NMR) spectroscopy. If it could be shown that TFT was concentrated in vivo in the tissues of HSV-2 infected animals as it is in vitro, it is possible that the excess accumulation could be detected noninvasively by $^{19}\text{F}$ NMR spectroscopy.

Because TFT crosses the blood brain barrier poorly, we measured TFT concentrations in the livers of mice with hepatitis due to HSV-2; for comparison, TFT levels were measured in the livers of uninfected mice, mice infected with the coronavirus MHV-A59, and mice with carbon tetrachloride (CCl$_4$) induced hepatitis.

Methods

Viruses used

The 333 strain of HSV-2 was kindly supplied by Dr. N. Balachandran, College of Veterinary Medicine, University of Florida, Gainesville, FL. HSV-2 was grown and titered in Vero cells as previously described (Rand, 1986).

MHV A59 was kindly supplied by Dr. Katherine Holmes, and was grown in 17CL-1 cells as described elsewhere (Holmes, 1986).

Mice

The following strains of mice were obtained from the National Cancer Institute: Balb/c, CBA/J, A/HeN, A/J, P/N, and DBA/2N. Preliminary studies showed that the CBA/J strain consistently yielded the highest titers in the liver following intraperitoneal infection of the 333 strain of HSV-2. All further studies were therefore done with CBA/J mice, aged 4–6 wk, weighing 18–23 g obtained from the Department of Pathology, University of Florida, Gainesville, FL and from Jackson Laboratories, Bar Harbor, ME.

Drugs

Trifluorothymidine was purchased from Sigma, St. Louis, MO. Carbon tetrachloride was obtained from Fisher (Springfield, NJ) and was dissolved in olive oil (Pompeian, Baltimore, MD) for injection. TFT was assayed by high pressure liquid chromatography (HPLC) by methods previously described (Rand, 1986).
Experimental design

Four to six week old, 18-23 g, CBA/J mice were injected intraperitoneally (i.p.) with 1.5 ml of HSV-2 (2-4 × 10^6 pfu/ml) or MHV A59 (≥ 10^6 pfu/ml). In this model, maximal HSV-2 titers are present in the liver between days 3 and 5, and mice generally died from day 4–6 from this high inoculum. Titers of HVM A59 are also maximal on days 3–5, but mice were less ill than when infected with HSV-2. Three days after infection, mice were anesthetized with a 3:1 mixture of 100 mg/ml ketamine HCl:20 mg/ml Xylazine at a dose of 0.2 ml/kg. The jugular vein was surgically exposed, and mice were injected intravenously with 100 or 160 mg/kg of TFT in the appropriate volume of a 20 mg/ml solution in Phosphate Buffered Saline (PBS) pH 7.4. We chose jugular venous injection because we could assure the accuracy of the intravenous injection. Mice were sacrificed at 45 min, 2, 2.5 and 3.5 h after I.V. injection of TFT. The livers were immediately excised and frozen at −70°C until they could be studied. For viral titration and measurement of TFT, 1.5 ml of PBS was mixed with 1 g of liver and the mixture homogenized (10 strokes using a Pyrex Ten Broeck tissue grinder), followed by sonication × 4 for 30 s in a Fisher 300 sonic dismembrator at a relative output of 0.55; 0.8 ml of the liver homogenate was mixed with an equal volume of methanol, vortexed extensively and then centrifuged at 15,000 × g for 15 min. The supernatant was analyzed by HPLC for TFT and the results expressed per gram of liver after correction for dilution. Prior to sonication, 0.5 ml of the liver homogenate was serially diluted in serum free tissue culture media and titrated in triplicate in Vero cells as previously described (Rand, 1986). In some experiments the clarified supernatant that had been mixed with methanol as described above was placed in a 5 mm NMR tube and 19F content measured by NMR spectroscopy (see below). In other experiments the entire liver was excised, and placed in a 12 mm NMR tube and the 19F directly analyzed by NMR spectroscopy (described below).

Carbon tetrachloride

Carbon tetrachloride was selected as a control for hepatitis because previous work suggested that the dose related hepatic toxicity was limited to the liver and did not involve the kidneys which might conceivably alter the pharmacokinetics of TFT (Watrous, 1972; Plaa, 1965; Klassen, 1966; Díaz Gómez, 1975). CCl₄ induces a dose dependent patchy, centrilobular necrosis which is analogous to that induced by HSV infection of the liver (Goodman, 1986). Prior to i.p. injection of CCl₄ in olive oil, 0.2 ml of blood was collected from each of a group of 5 mice. Twenty-four hours after i.p. injection of 0.2 ml/kg CCl₄ in olive oil, blood was collected again, and both sets were analyzed for Alanine Amino Transferase (ALT) and Blood Urea Nitrogen (BUN). For ALT, 2 μl serum was analyzed in duplicate with a Technicon RA 1000 and for BUN 10 μl duplicate samples were analyzed with a Beckman Astra 8. Mice were sacrificed by cervical dislocation and the liver and kidney fixed in 10% formalin, stained with hematoxylin and eosin and examined histologically.

Nuclear magnetic resonance spectroscopy

19F assay was carried out in a Nicolet-GE NT-300 NMR spectrometer, using
standard techniques with a simple one-pulse sequence. The field was 7.05 Tesla (T) and the frequency was 282 MHz. Depending upon the volume of the sample available, it was placed in either a 5 mm or 12 mm diameter tube, which was usually not spun. Both whole liver and liver homogenates were placed in ≈ 2 ml of a 2:1 v/v mixture of D₂O:methanol which inactivated HSV, permitted field homogeneity to be optimized and provided field-frequency lock for time averaging. A 5-s cycle time was used with a tip angle of 60°. Sweep width was 5000 Hz and 64K data points were collected. Concentration of ¹⁹F in a sample was evaluated by comparing the electronically integrated area of the resonance peak, after application of 10 Hz line broadening and Fourier transformation of the free induction delays using a 1280 computer, to that for a standard containing 40, 50, or 100 μg/ml. Instrumental reproducibility of the calculated areas was checked by comparing the integral for the 40 μg/ml standard to that calculated from a scale setting obtained with a 100 μg/ml standard. In the calculation of TFT concentration, appropriate allowances were made for the dilution of the sample or for the size of the organ in the sample tube, as well as for the TFT extracted from the organ by the surrounding medium.

Statistics

Because of the very wide variation in TFT levels and their relationship with viral titers at ≥ 10⁶ pfu/g liver, we could not assume that TFT levels were normally distributed among HSV infected mice. Therefore, data were analyzed by the non-parametric Mann-Whitney U test.

Results

Table 1 shows the results of a representative experiment in which TFT levels and viral titers were measured in the livers of HSV-2 infected and uninfected 4–6 wk old CBA/J mice. Infected mice had a TFT mean ± SE of 110.1 ± 52.7 μg/g liver, compared with 14.7 ± 7.7 μg/g liver for uninfected mice, P = 0.014, Mann-Whitney U.

| INFECTED | UNINFECTED |
|-----------|-------------|
| Viral titer (PFU/g liver) | Conc. of TFT (μg/g liver) | Conc. of TFT (μg/g liver) |
| 1.1 × 10⁷ | 266.6 | 36.5 |
| 2.4 × 10⁸ | 60.2 | 2.5 |
| 1.1 × 10⁹ | 74.6 | 14.3 |
| 3.4 × 10¹ | 38.9 | 5.6 |

Mean*  

| INFECTED | UNINFECTED |
|-----------|-------------|
| Mean* | 110.1 | 14.7 |

* P = 0.014 Infected vs. Uninfected, Mann-Whitney U. see Materials and Methods for explanation.
At a dose of 0.2 ml/kg CCl₄ in olive oil injected i.p., overwhelming hepatic necrosis resulted a day later, with ALT levels in blood in the range of 18,000–28,000 IU/ml (see Table 2). As shown by the BUN, there was no significant alteration in renal function. Histologically, there was a dose related centrilobular necrosis, which at 0.2 ml/kg was massive, but at 0.02 ml/kg was more localized and less extensive. Preliminary experiments were then carried out to determine the average ALT levels of HSV-2 infected mice on day 3 at the time higher levels of TFT were found in the liver. On day 3 following i.p. injection with 4–6 × 10⁶ pfu HSV-2, 4 mice had an ALT mean ± SD of 3554 ± 781 IU/ml blood. A dose response curve of CCl₄ hepatitis showed that between 0.015–0.02 ml/kg CCl₄ would lead to ALT levels of 500–5000 IU/ml blood 24 h later. Therefore, CCl₄ was used at a dose of 0.02 ml/kg. Despite the preliminary data, the ALT levels measured in the blood of the 12 CCl₄ treated mice actually given TFT was 18,950 ± 7,526 (Table 2).

Fig. 1 shows that there was essentially no difference in blood levels of TFT at 45 min after i.v. injection, and no measurable blood levels of TFT at 2 h among any of the groups. HSV-2 infected mice had significantly higher levels of TFT in their livers at both 2 and 3.5 h after i.v. injection of TFT, whether compared with uninfected or CCl₄ treated mice.

If TFT levels in liver were due to accumulation in HSV infected liver cells, then the higher the HSV titer/g liver, the higher the TFT level should be. The relationship between HSV-2 pfu/g liver and TFT concentration/g liver among infected mice is illustrated in 2 separate experiments in Fig. 2. The first experiment (○'s) shows the relationship between HSV-2 titer in pfu/g liver and TFT concentration/g liver for the 9 HSV-2 infected mice studied at 2 h after i.v. injection of TFT shown in Fig. 1. Linear regression showed a correlation coefficient of \( r = 0.72, \) \( P < 0.05 \) in this experiment (○'s) and \( r = 0.99, \) \( P < 0.05 \) in the other (●'s). A similar relationship was also observed among infected mice studied at 3.5 h after receiving

**Table 2**

Blood urea nitrogen and serum alanine aminotransferase levels in carbon tetrachloride and herpes simplex virus-2 treated CBA/J mice.

| Treatment              | Mean ± SD             |
|------------------------|-----------------------|
|                        | BUN\(^a\) (mg/dl)     | ALT\(^b\) (IU/L) |
| **CCl₄**               |                       |
| Pre-treatment (N=5)^c  | 10.4 ± 2.7            | 28.2 ± 13.3       |
| 24 h post-treatment (N=5)^c | 6.4 ± 2.3            | 28112 ± 6086      |
| 24 h post-treatment (N=12)^d | ND                  | 18950 ± 7526     |
| **HSV-2**              |                       |
| 48 h post-infection    | ND                    | 500               |
| 72 h post-infection (N=4) | ND                  | 3554 ± 781       |

\(^a\) 10 μl serum, BUN measured with the Beckman Astra 8. \(^b\) 2 μl serum, ALT measured with the Technicon RA 1000. \(^c\) Mean ± SD of the BUN and ALT prior to CCl₄ treatment and from the same mice 24 h later, CCl₄ used at 0.2 ml/kg i.p. \(^d\) Mean ± SD of the 12 CCl₄ treated mice shown in Fig. 1. 45 min (N=4), 2 h (N=4) and 3.5 h (N=4) after receiving 100 mg/kg TFT, CCl₄ used at 0.02 ml/kg. ND = Not done.
Fig 1. HSV-2 infected, uninfected and CCl4 treated 4-6 wk old CBA/J mice were injected via the jugular vein with 100 mg/kg TFT, and sacrificed 45 min, 2 h or 3.5 h later. The figure shows the mean ± SE of the TFT concentration in blood or liver measured by HPLC as described in Methods. Most of the experiments were done on day 3 after HSV infection; day 2 after infection was not systematically studied, and is presented only for comparison.

Fig. 2. Relationship between HSV-2 titer/g liver and TFT concentration/g liver measured by HPLC on day 3 after HSV-2 infection, 2 h (O) and 2.5 h (●) following i.v. administration of TFT. Two separate experiments are shown. Data were analyzed by linear regression. The 9 HSV-2 infected mice (O) are the same mice shown in the bar graph (Fig. 1) at 2 h. For comparison, TFT levels in the livers of the 9 uninfected mice used in these 2 experiments were pooled and the mean ± SD shown.
TABLE 3
TFT concentration (µg/g liver) measured by HPLC and $^{19}$F NMR 2 h after intrajugular injection of 100 mg/kg TFT in CBA/J mice.

|                  | TFT µg/g liver | HPLC<sup>b</sup> | NMR<sup>c</sup> |
|------------------|----------------|-----------------|-----------------|
|                  | Viral titer<sup>a</sup> |                 |                 |
| HSV-2 infected    |                |                 |                 |
| 1                | $7.5 \times 10^6$ | 152.1           | 90              |
| 2                | $6.6 \times 10^6$ | 149.1           | 182.5           |
| 3                | $6.1 \times 10^6$ | 38.9            | 25              |
| 4                | $3.6 \times 10^6$ | 63.6            | 55              |
| Uninfected       |                |                 |                 |
| 1                | N/A            | 16.0            | 45              |
| 2                | N/A            | 3.5             | 0               |
| 3                | N/A            | 13.8            | 20              |
| 4                | N/A            | 9.9             | tr              |
| CCl<sub>4</sub> hepatitis |            |                 |                 |
| 1                | N/A            | <1.0            | tr              |
| 2                | N/A            | <1.0            | tr              |
| 3                | N/A            | <1.0            | 0               |
| 4                | N/A            | <1.0            | 0               |

<sup>a</sup> pfu/g liver. <sup>b</sup> Correlation coefficient, HPLC vs. NMR, $r = 0.91$, $P < 0.0005$. <sup>c</sup> Calculated from the area under peak at $-63$ ppm (relative to CFCl<sub>3</sub>).

TFT as well as those receiving the higher dose of 160 mg/kg TFT shown in Table 1.

In a separate experiment, TFT levels and HSV titers were measured using a small portion of the liver, leaving approximately 1 g intact from 4 mice/group to measure $^{19}$F levels by NMR spectroscopy, under conditions as described in the methods. The results are shown in Table 3, compared with the HSV pfu/g liver where applicable and the TFT levels as measured by HPLC. By linear regression, the correlation between HPLC and NMR was $r = 0.91$, $P < 0.0005$.

Fig. 3 shows actual $^{19}$F NMR tracings of (A) the 40 µg/ml standard, (B) liver homogenate from an HSV-2 infected mouse 3.5 h after i.v. injection of 100 mg/kg TFT; the area under the peak corresponds to 11 µg/g liver, (C) liver homogenate from a CCl<sub>4</sub> treated mouse and (D) liver homogenate from an uninfected mouse 3.5 h after i.v. injection of 100 mg/kg TFT.

As a further specificity control, 10 CBA mice were infected with murine hepatitis virus (MHV-A59), a coronavirus which does not contain thymidine kinase. As shown in Fig. 4, there was no increased concentration of TFT in livers of 10 MHV infected mice 2 h after i.v. injection of TFT, compared with the levels found in uninfected mice in the same experiment. In contrast, the 2 HSV-2 infected mice with titers of $10^6$ pfu HSV/g liver, had strikingly elevated levels.
Fig. 3. Representative $^{19}$F NMR spectra measured with the Nicolet GE NT-300 NMR spectrometer at a field strength of 7.05 T, using a 2:1 v/v $D_2O$/methanol mixture as a field-frequency lock. (A) 40 µg/ml TFT standard, acquisition time ≈7 min. (B) HSV-2 infected mouse, day 3, 3.5 h after i.v. administration of 100 mg/kg. Liver homogenate prepared as described in Methods. Acquisition time was ≈28 min, and TFT concentration was 11 µg/g liver by NMR. (C) CCl₄ treated mouse, 3.5 h after i.v. administration of 100 mg/kg TFT, liver homogenate, prepared as described in the Methods. Acquisition time was 1.9 h, which accounts for the low noise level. (D) Uninfected mouse 3.5 h after i.v. administration of 100 mg/kg TFT, liver homogenate prepared as in the Methods. Acquisition time ≈28 min. Horizontal line in A and B represents the cumulative area under the curve.

Fig. 4. Infection of CBA/J mice with MHV-A 59 showing levels of TFT in the same range as that of uninfected mice. No relationship between MHV titer and TFT level was observed. As a positive control, other CBA/J mice were infected with HSV-2 at the same time as those infected with MHV-A 59 and those infected with HSV-2 in the range of $10^6$ pfu/g liver again showed high levels of TFT in liver. Symbols used: ●=HSV-2; ○=MHV-A 59; △=uninfected.
Discussion

Significantly higher levels of TFT were observed in the livers of HSV-2 infected mice, as compared with those of uninfected mice, mice infected with MHV or mice treated with CCl₄. The effect was repeatedly demonstrated, and correlated with the level of HSV-2 infection. Since blood levels of TFT among HSV-2 infected mice were similar to or even lower than those of uninfected mice or CCl₄ treated mice, it seems unlikely that delayed excretion or otherwise altered pharmacokinetics could account for the higher levels of TFT observed in the HSV-2 infected livers. Non-specific accumulation due to hepatic damage seems unlikely as well in view of the results in MHV or CCl₄ hepatitis.

If TFT accumulation were specifically due to phosphorylation by the HSV-2 induced thymidine kinase, we would expect to find the mono-, di-, and triphosphorylated intermediates in liver tissue. However, phosphorylated nucleosides (such as adenosine triphosphate) are degraded so rapidly to the corresponding nucleosides after cessation of blood flow, or hypoxia, that measurement of these intermediates is impossible unless made in vivo (Clouse, 1985) or after snap freezing tissues in liquid N₂ followed by chemical analysis or high resolution ³¹P NMR (Ghonek, 1982). Under the conditions of the HPLC measurements we performed, phosphorylated intermediates would not have been detectable. Radioactive TFT was not available, so that microscopic autoradiographic correlation with HSV antigen positive cells was not possible.

Data from the literature provide support both for and against HSV specific concentration of antiviral drugs in HSV infected tissues. Price et al. (1983, 1986) and Saito et al. (1982, 1984) using ¹⁴C labeled 2'-fluoro-5-methyl-1-β-D-arabinosyluracil (FMAU) were able to show specific concentration of the drug in the optic nerve and chiasm of rats after intraocular inoculation of HSV-1. However, there was also non-specific accumulation of FMAU in the choroid plexus and the cells lining the ventricular walls. Biron et al. (1982) studied ¹⁴C labeled acyclovir, which has been shown to accumulate intracellularly in HSV infected cells in vitro (Furman, 1981). Following s.c. injection, acyclovir levels were similar in all HSV infected and uninfected tissues studied, i.e. liver, kidney, spleen, lung, blood and brain. Price et al. (1986) were also unable to demonstrate any selective accumulation of acyclovir in HSV infected tissues in vivo. It seems difficult to reconcile the lack of accumulation of acyclovir in HSV infected tissues, with our observation of high levels of TFT in livers from HSV-2 infected mice, since both compounds are phosphorylated by the HSV TK and do accumulate in tissue culture.

Recently, Smee et al. showed that while acyclovir accumulated in the form of phosphorylated intermediates in HSV infected cells in vitro, it was also rapidly degraded back to acyclovir, and diffused out of the cell (Smee et al., 1985). In contrast, DHPG also accumulated in a similar manner, but did not hydrolyze back to the parent compound as readily. Thus, even related drugs which utilize the same basic steps in their mechanism of action can behave quite differently biologically. Furthermore, we used i.v. injection of TFT via the jugular vein, which would result in extremely high blood levels initially, with a rapid fall off, as we observed.
i.e. no detectable drug at 2 h in the blood. When we gave TFT by i.p. injection, we were unable to demonstrate accumulation of TFT in the liver of HSV infected mice. Since Biron et al. (1982) used s.c. injection, and since plasma levels remained reasonably elevated for 2 h or more after injection, it is possible that the 'depot' effect of s.c. injection, together with the propensity of phosphorylated acyclovir to be rapidly hydrolyzed back to the parent compound, was responsible for the lack of accumulation. Another difference between our experiments and those of Biron et al. (1982) is that we measured drug levels on day 2 or 3 after infection, while their drug studies were carried out on days 4–5 after infection. It is very possible that the relationship between drug accumulation and level of infection changes during the course of the infection, and some preliminary observations in this regard are discussed below.

Nuclear magnetic resonance spectroscopy was used to measure levels of $^{19}$F. Since there is essentially no tissue background level of fluorine, the area under the curve could be directly related to a standard and used to estimate tissue levels of TFT. TFT levels as low as 11 µg/g tissue were readily detected, and even at the 1:6 dilution used, required only 28 min acquisition time, albeit at a high field strength of 7.05 T. Although the highest field strengths used diagnostically in humans are about 2 T, $^{19}$F NMR surface coil technology may well have sufficient sensitivity in this range of field strength. For example, a Biospec system operating at 94 MHz was used to investigate metabolites of 5-fluorouracil (5 FU), and had a detection limit of 0.1 µmol/g in a mouse liver and a mouse tumor, with 10–20 min acquisition times (manufacturer's technical information). Wolf et al. (1986) reported similar studies of the behavior of 5 FU in human liver, obtaining suitable spectra in 8 min in a field of 1.5 T. While the transition from spectroscopy to surface coil configuration as required for the study of living organisms may pose problems, it would probably not result in much loss of sensitivity.

One interesting finding was that the higher levels of TFT could be found in the livers of HSV-2 infected mice on day 2 as well as day 3 following HSV-2 infection. Here, the viral titers were quite low ($10^3$/g liver) compared with those in mice demonstrating the TFT accumulation on day 3 ($\geq 10^6$ pfu/g liver). ALT levels in blood in mice 2 days after HSV infection were also much lower, compared with day 3, and were in the range of 500 IU/ml blood; but data were only available from a small number of animals. Since the HSV induced TK is maximally produced approximately between 7–15 h after infection (Kit, 1975; Fong, 1980), which is before release of infectious virus, it is possible that early in the course of infection in vivo, as virus is spreading rapidly and infecting ever increasing numbers of new cells, higher levels of TK are present relative to the number of infectious virions, resulting in greater uptake of TFT per infectious unit.

Proof that TFT accumulation was due specifically to the induction of TK by HSV-2 would require the direct measurement of phosphorylated TFT. HSV-2 mutants which do not induce TK could be used as a control, but are generally less virulent than wild type HSV (Coen, 1982). Since the level of TFT achieved is dependent on viral titer, such TK negative strains would have to replicate to titers equivalent to those of the wild type in vivo for results to be interpretable.
In summary, highly elevated levels of TFT were observed in livers of HSV-2 infected mice compared with either uninfected mice, mice infected with MHV-A59 or CCl₄ treated mice. There was good correlation between TFT levels in liver tissue and HSV-2 titers in the same tissues. Neither altered pharmacokinetics, nor non-specific liver damage could account for the observed drug accumulation. NMR spectroscopy has the sensitivity to detect high levels of TFT readily, and thus offers a potentially non-invasive method for the diagnosis of visceral HSV infection. Because TFT does not readily cross the blood brain barrier, this methodology may not be directly applicable to the diagnosis of HSV encephalitis. However, visceral infection particularly involving the liver by HSV-2 is well described in immunocompromised (Berglin, 1982; Taylor, 1981; Walker, 1981; Elliott, 1980; Holdsworth, 1976), as well as non-immunocompromised patients (Díaz Gómez, 1975; Anuras, 1976; Connor, 1979; Eron, 1976; Manoux, 1983; Joseph, 1974; De Berardinis, 1983). In addition, neonates with disseminated HSV-2 infection may not have clinically obvious skin vesicles, oral ulcers or keratitis (Moedy, 1981), and still have liver involvement. Studies of TFT accumulation needed before clinical application can be tested.

Acknowledgement

We gratefully acknowledge the excellent technical assistance of Laszlo Prokai, Ph.D.

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