

Effect of 2'-fluoro-5-methyl-ara-uracil and cyclophosphamide on herpes simplex virus infection in guinea pigs

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ABSTRACT Ip 2'-fluoro-5-methyl-arauracil (FMAU) 50 (mg/kg)/d for 3 d starting 1 d post-vaginal inoculation of HSV-2 almost completely inhibited the appearance of primary genital lesions (GL) but did not reduce the establishment of HSV latency. When ip cyclophosphamide (Cy) 50(mg/kg)/d for 7 d was simultaneously given, mild delayed GL were occasionally noticed in FMAU-treated animals. During HSV-2 latent infection, ip Cy 50 (mg/kg)/d for 10 d could induce genital herpes recurrence of FMAU-treated animals. Ip Cy 300 (mg/kg)/dose for 3 doses at

weekly intervals could increase the incidence of recurrent GL, suggesting that the reactivation of latent HSV by Cy was dosage related.

KEY WORDS 2'-fluoro-5-methyl-arabinofuranosyluracil; herpesvirus hominins; guinea pigs; drug therapy

FMAU (2'-fluoro-5-methyl-ara-uracil) or 1-(2'-fluoro-2'-deoxy- β -D-arabinofuranosyl) thymine was synthesized by Watanabe *et al*⁽¹⁾ and showed potent antiherpes simplex virus activity in cell cultures⁽²⁾, encephalitis infections in mice⁽³⁾, acute keratitis in rabbits⁽⁴⁾, and primary acute genital herpes (GH) in guinea pigs⁽⁵⁾. However, it is unknown whether FMAU treatment of primary GH in guinea pigs can reduce or prevent the establishment of latent infection. This paper reports that FMAU, as other

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antiviral agents, could not eradicate HSV latency from nerve tissues even though treated guinea pigs did not show clinical lesions during acute infection.

MATERIALS AND METHODS

Virus and cells HSV-2 strain 1868 was originally isolated in our laboratory⁽⁶⁾. Virus stock was prepared in primary guinea pig embryo (GPE) cells and infectivity titers were determined by plaque formation in GPE cells.

Animals and virus inoculation Young Hartley guinea pigs were inoculated intravaginally with $10^{4.8-5.2}$ PFU of HSV-2 strain 1868 and the genital lesions (GL) were scored⁽⁷⁾.

Virus recovery from guinea pig vaginas Guinea pig vaginas were swabbed with pre-moistened cotton-tipped applicators which were then placed in a vial with 1.0 ml of minimal essential medium (MEM) containing Earle's balanced salt solution (EBSS), 2% calf serum, and 10% (vol/vol) dimethylsulfoxide. All samples were frozen at -70°C until plaque assay in GPE cells.

FMAU treatment FMAU and drug vehicle solutions were kindly provided by Dr C McLaren (Bristol-Myers Co). FMAU was weighed and dissolved in the drug vehicle solution (20% propylene glycol, 20% ethanol and 60% water) at a concentration of 50 mg/ml. Then, the FMAU solution was sterilized by passing through a membrane filter. Guinea pigs were treated with ip FMAU twice daily at 9 am and 4 pm for 3 d. Sham-treated groups received drug vehicle solution only.

Cyclophosphamide treatment Cyclophosphamide (Cy) was purchased from Sigma Chemical Co and dissolved in PBS at a concentration of 50 mg/ml. The drug solution was sterilized with a $0.22\ \mu\text{m}$ millipore membrane filter and stored at 4°C .

In acute infection experiments, two groups of guinea pigs were treated ip with

a single dose of Cy 50(mg/kg)/d for 7 d and FMAU was added to one group simultaneously. For latent infection experiments, animals were infected and treated with FMAU ip during acute infection. Approximately 30-183 d later, half of the FMAU treated and half of the untreated groups received Cy 50 (mg/kg)/d for 10 d. The other half of each group received PBS ip only. In addition, several untreated animals were given Cy 50 (mg/kg)/d for 10 d or three doses of 300 (mg/kg)/dose at weekly intervals to determine if there was a dosage response to Cy reactivation. All animals were checked daily for recurrent lesions and virus shedding in the vagina was monitored every three days for 28 d.

Isolation of latent virus from spinal cord and ganglia All animals with and without recurrent herpes were sacrificed at the end of each experiment. Spinal cord (SC) and lumbosacral dorsal root ganglia (DRG) were removed aseptically, minced in MEM containing EBSS and 2% calf serum, then cocultivated with GPE cells for 35-40 d. To enhance the recovery of latent virus from the nerve tissues, each of the cocultivated tissues was transferred to a fresh monolayer culture every 7-10 d or until virus induced CPE was observed. All of the recovered viruses from nerve tissues were typed by the methods described⁽⁸⁾.

RESULTS

Effect of FMAU on guinea pig GL during acute infection Four groups were treated with FMAU starting 1, 2, 3, or 4 d postinfection in order to investigate the effect of initiating FMAU at different times. As shown in Fig 1, FMAU treatment starting 1 or 2 d postinfection significantly inhibited the development of GL in guinea pigs. The result of treatment starting 3 or 4 d postinfection was not statistically different from sham-treatment.

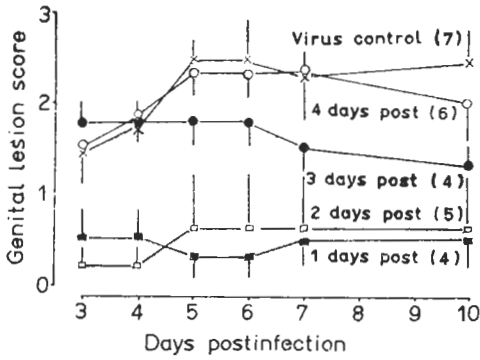


Fig 1. Effect of 2'-fluoro-5-methyl-ara-uracil (FMAU) 12.5 (mg/kg)/d \times 3 d on genital lesions (GL) of guinea pigs. The GL scores of 1 or 2 d postinfection treatment were significantly less than 3 or 4 d postinfection treatment and sham-treated guinea pig controls: $p < 0.01$ on d 3 and 7. There was no difference between 3 and 4 d postinfection treatment or sham-treated guinea pig controls: $p > 0.05$ on d 5. Number of animals in parentheses. Virus inoculation was $10^{4.8}$ PFU. $\bar{x} \pm SD$.

Effect of FMAU and Cy on GL and virus shedding during acute infection GL in FMAU-treated guinea pigs starting 1 d postinfection were completely inhibited for 7 d (Fig 2). Similar to the finding stated above, significant differences in mean GL scores were found between FMAU-treated and sham-treated groups. However, the two groups of animals that received Cy for 7 d had more GL than the comparable nonsuppressed groups regardless of whether they were treated with FMAU or not.

On d 5 to 7 after virus inoculation,

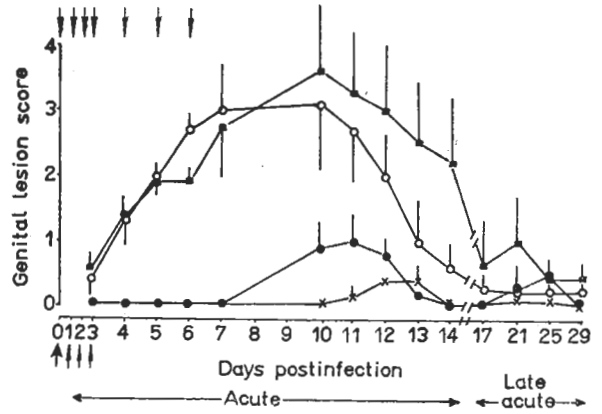


Fig 2. Effect of FMAU and cyclophosphamide (Cy) on genital lesions (GL) during acute infection. 8 guinea pigs/group. GL scores between groups on d 11 postinfection: FMAU vs control $p < 0.01$; FMAU vs FMAU + Cy $p < 0.05$; FMAU + Cy vs control $p < 0.05$. (●) FMAU+Cy; (○) control; (■) Cy; (×) FMAU; ↓ Cy 50 (mg/kg)/d; ↑ Virus inoculation ($10^{5.2}$ PFU); † FMAU 50 (mg/kg)/d. $\bar{x} \pm SD$.

the mean virus titers from the genital swabs of FMAU-treated animals were about one log less than the titers from the sham-treated animals and Cy-treated guinea pigs (Tab 1). Excretion of virus in the genital tracts by animals which received FMAU plus Cy was also inhibited on d 5 postinfection, but returned to the virus titers on the average in control animals on d 7 postinfection.

Cy reactivation of GH during latent infection The results are shown in Tab 2.

Tab 1. Effect of 2'-fluoro-5-methyl-ara-uracil (FMAU) and cyclophosphamide (Cy) on virus shedding from the vagina of guinea pigs infected with HSV-2 ($10^{5.2}$ PFU/0.1 ml). $n = 8$. $\bar{x} \pm SD$

| Drugs | Virus titer (log PFU/0.1 ml) after infection | | | | |
|---------|----------------------------------------------|---------------|---------------|---------------|---------------|
| | d 3 | d 5 | d 7 | d 10 | d 13 |
| Control | 2.7 \pm 1.5 | 2.4 \pm 0.5 | 1.6 \pm 0.6 | 0.5 \pm 0.8 | 0 |
| FMAU | 3.2 \pm 1.0 | 1.1 \pm 1.2 | 0.7 \pm 1.1 | 0.4 \pm 1.1 | 0 |
| FMAU+Cy | 2.5 \pm 1.0 | 0.2 \pm 0.6 | 1.8 \pm 1.3 | 0.2 \pm 0.6 | 0 |
| Cy | 3.3 \pm 1.4 | 2.4 \pm 1.1 | 1.7 \pm 0.7 | 0.3 \pm 0.8 | 0.5 \pm 1.3 |

On d 5: FMAU vs control and Cy, $p < 0.05$; FMAU+Cy vs Cy and control, $p < 0.01$.

On d 7: FMAU vs Cy, $p < 0.05$.

Tab 2. Genital lesion recurrence of latently infected guinea pigs following cyclophosphamide (Cy) administration. * $p > 0.05$, * $p < 0.01$**

| Drugs received | | Guinea pigs | |
|----------------|--------|-------------|-------------------|
| Acute | Latent | Tested | Recurrence |
| FMAU | Cy | 13 | 12 ₇ * |
| — | Cy | 8 | 5 ₁ ** |
| FMAU | PBS | 11 | 4 ₁ * |
| — | PBS | 6 | 2 |

The percentage of FMAU-treated animals with recurrent GL was significantly higher in the Cy group than in PBS controls. Some of the guinea pigs treated with 12.5 (mg/kg)/d or 50 (mg/kg)/d of FMAU and showed no clinical GL during acute infection still developed recurrent GL. Virus shedding from the vagina was seldom found during the recurrent period. One of the sham-treated, Cy-reactivated guinea pigs had virus recovered from the vagina following GL recurrence 11 d after Cy treatment was started. Another sham-treated animal in the PBS group had vaginal virus shedding 27 d after Cy treatment. At that time, this guinea pig had no apparent GL. During the 28 d observation, one or two tiny GL on the external genital skin were noticed. Most of the recurrent GL appeared for only 1 or 2 d although additional GL occurred in sequence for a period of 3-4 d (Fig 3). The earliest GL appeared 4 d after Cy treatment was started.

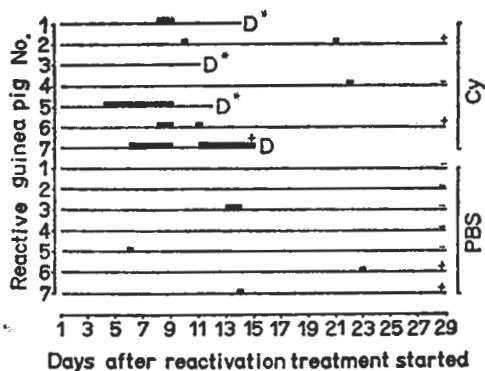


Fig 3. Effect of cyclophosphamide on genital lesions (GL) recurrence: Time course of recurrent GL of HSV latently infected guinea pigs which were treated with FMAU during acute infection. Bars represent the duration of recurrent GL. D=Dead, *virus isolation was not attempted, + virus was isolated from spinal cord &/or dorsal root ganglia.

Tab 3. Recovery of herpes simplex virus from nerve tissues during latent infection following cyclophosphamide (Cy) treatment. All guinea pigs were killed on or after 58 d postinfection. ** $p < 0.05$

| Drugs received | | Guinea pigs | |
|----------------|--------|-------------|----------------------|
| Acute | Latent | Tested | Virus in SC &/or DRG |
| FMAU | Cy | 4 | 3 ₁ |
| — | Cy | 8 | 6 ₁ * |
| FMAU | PBS | 11 | 3 ₁ * |
| — | PBS | 6 | 3 ₁ ** |

Virus recovery from nerve tissues after Cy reactivation A total of 29 animals examined for recurrent GH were sacrificed

Tab 4. Effect of cyclophosphamide (Cy) in the reactivation of genital herpes (GH).

| Dosage | Number of doses | Number Tested | Guinea pigs showing first GH on d after Cy initiation | | | | | Total |
|-------------------------|-----------------|---------------|-------------------------------------------------------|-------|--------|---------|---------|-------|
| | | | d 1-2 | d 3-7 | d 8-14 | d 15-21 | d 22-28 | |
| 300 mg/(kg·wk) | 3 | 11 | 4 | 4 | 0 | 0 | 0 | 8 |
| 50 mg/(kg·d) | 10 | 12 | 0 | 2 | 4 | 2 | 0 | 8 |
| PBS control (1 ml/d) | 10 | 12 | 0 | 2 | 1 | 2 | 0 | 5 |

at least 4 wk after starting Cy treatment. Infectious HSV-2 from SC/DRG was recovered from 9/12 Cy and 6/17 PBS treated animals by cocultivation (Tab 3). However, compared to sham-treated animals, FMAU treatment during acute infection did not significantly reduce the percentage of animals from whom latent HSV-2 was isolated by cocultivation.

Comparison of high and low dosages of Cy in the reactivation of GH As shown in Tab 4, with 50 mg/(kg·d), 8/12 animals developed GL with an average 3 d duration. There were 2/12 deaths. With 300 mg/(kg·wk), 8/11 had GH which appeared 1-2 d after Cy initiation and persisted for a longer period (average, 6 d). The same GL were not necessarily present for many days, rather in some instances one GL resolved and another appeared. In addition, with the higher dosage of Cy, there was a higher mortality with 4 out of 11 animals dying. HSV-2 was not isolated from these recurrent GL.

DISCUSSION

A previous study showed that FMAU had the most potent effect on the treatment of GH in guinea pigs when compared with acyclovir, phosphonoformate, 1-(2'-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytidine, and 1-(2'-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil⁽⁶⁾. The results obtained from the current study confirmed the previous findings. However, guinea pigs treated with FMAU plus Cy showed less virus shedding from vagina than both FMAU treated and Cy alone treated animals on d 5 postinfection. This observation means that, on d 2 following FMAU treatment end, the vaginal virus shedding from animals simultaneously receiving Cy could still be reduced due to the remains of FMAU effect. On d 4 of FMAU treatment end, i. e. 7 d postinfection, animals receiving FMAU plus Cy were immunosuppressed. Therefore, more

virus was shed. Although Cy treatment appeared to enhance the appearance of GL in FMAU-treated guinea pigs during acute infection, the drug treated animals showed comparatively fewer GH than those animals without FMAU treatment. Thus, FMAU therapy even in immunosuppressed guinea pigs was successful in reducing the severity of GH infection.

Several studies have been concerned with experimental reactivation of latent HSV by various means⁽⁸⁾ including immunosuppressive agents. Hydrocortisone⁽⁹⁾ and Cy⁽¹⁰⁾ have been applied to reactivate HSV in the trigeminal ganglia. To date none of these studies have been applied to evaluate herpes recurrence in drug-treated animals. In the present study, we show that Cy can reactivate HSV during latent infection, resulting in a higher incidence of GH recurrences and a higher rate of latent virus recovery from nerve tissues. With Cy treatment, we demonstrate that FMAU starting 1 d postinfection did not reduce the establishment of HSV latency in the nervous tissues of guinea pigs although clinically FMAU prevented GH formation during acute infection. The findings that FMAU did not influence the establishment of HSV latency were not surprising since HSV-2 can reach the sensory ganglia within 1-2 d after infection⁽¹¹⁾.

REFERENCES

- 1 Watanabe KA, Reichman U, Hirota K, Lopez C, Fox JJ. Nucleosides 110. Synthesis and antiherpes virus activity of some 2'-fluoro-2'-deoxyarabinofuranosylpyrimidine nucleosides. *J Med Chem* 1979; 22 : 21
- 2 Fox JJ, Lopez C, Watanabe KA. 2'-fluoro-arabinosylpyrimidine nucleoside: Chemistry, antiviral, and potential anticancer activities. In: De las Heras FG, Vega S, eds. *Medicinal Chemistry Advances*. 1st ed. NY. Pergamon Press, 1981 : 27-40
- 3 Schinazi RF, Peters J, Sokol MK, Nahmias AJ. Therapeutic activities of 1-(2-fluoro-2-

- deoxy- β -D-arabinofuranosyl)-5-iodocytosine and thymine alone and in combination with acyclovir and vidarabine in mice infected intracerebrally with herpes simplex virus. *Antimicrob Agents Chemother* 1983; 24 : 95
- 4 Trousdale MD, Nesburn AB, Su TL, Lopez C, Watanabe KA, Fox JJ. Activity of 1-(2'-fluoro-2'-deoxy- β -D-arabinofuranosyl) thymine against herpes simplex virus in cell culture and rabbit eyes. *Ibid* 1983; 23 : 808
 - 5 Mayo DR, Hsiung GD. Treatment of primary acute genital herpes in guinea pigs by intraperitoneal administration of fluoropyrimidines. *Ibid* 1984; 26 : 354
 - 6 Zheng ZM, Mayo DR, Hsiung GD. Comparison of biological, biochemical, immunological, and immunochemical techniques for typing herpes simplex virus isolates. *J Clin Microbiol* 1983; 17 : 396
 - 7 Zheng ZM, Mayo DR, Hsiung GD. Effect of CP-20, 961 on genital herpes in guinea pigs. *Antiviral Res* 1983; 3 : 275
 - 8 Hurd J, Robinson TWE. Herpes virus reactivation in a mouse model. *Antimicrob Agents Chemother* 1977; 3 (Suppl A) : 99
 - 9 Underwood GE, Weed SD. Recurrent cutaneous herpes simplex in hairless mice. *Infect Immun* 1974; 10 : 471
 - 10 Kurata T, Kurata K, Aoyama Y. Reactivation of herpes simplex virus (type 2) infection in trigeminal ganglia and oral lips with cyclophosphamide treatment. *Jpn J Exp Med* 1978; 48 : 427
 - 11 Stanberry L, Kern ER, Richards JT, Abbott TM, Overall JC Jr. Genital herpes in guinea pigs: Pathogenesis of the primary infection and description of recurrent disease. *J Infect Dis* 1982; 146 : 397

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2'-氟-5-甲基-阿糖-尿嘧啶和环磷酰胺对豚鼠单纯疱疹病毒感染的影响

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提要 豚鼠阴道内接种 HSV-2 后, ip FMAU 50 mg/(kg·d) × 3 d 几乎完全抑制原发性生殖器疱疹 (GH) 出现, 但不能阻止 HSV 在神经组织建立潜伏感染 (LI)。同时 ip Cy 50 mg/(kg·d) × 7 d 时, 偶见少数豚鼠有延迟的 GH 出现。在 HSV-2 LI 期, ip Cy

50 mg/(kg·d) × 10 d 可在急性感染期先用 FMAU 处理的豚鼠发生复发性 GH (12/13), ip Cy 300 mg/kg, 共 3 次, 每次间隔 1 wk, 可加速 GH 复发, 提示潜伏的 HSV 激活与 Cy 剂量有关。

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关键词 2'-氟-5-甲基-阿糖尿嘧啶, 人疱疹病毒, 豚鼠, 药物疗法