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Foscarnet Inhibits Differentiation of Osteoblasts in Vitro. L. Titus, S.D. Boden, G. Hair, M. Racine, and R.F. Schinazi. VAMC and Emory University School of Medicine, Atlanta GA 30033

Foscarnet (PFA) is a pyrophosphate analog used primarily to treat cytomegalovirus infections in AIDS patients. This drug causes osteomalacia and failed mineralization of bone in cats after two weeks of i.v. infusion. Although systemic effects of PFA undoubtedly contribute to these bone lesions, the data suggest that the failure of mineralization reflects direct effects of PFA on the osteoblast. We present here data that at a nontoxic dose (similar to plasma levels in treated patients) PFA dose-dependently blocks rat preosteoblast differentiation in cell culture. Preosteoblasts from fetal rat calvariae differentiate in culture to mature osteoblasts when stimulated for seven days with 1 nM glucocorticoid (Triamcinolone). Osteoprogenitor cells within these cultures form nodules which deposit hydroxyapatite and secrete the bone specific matrix protein, osteocalcin. The progression of differentiation can be determined by monitoring expression of specific genes whose expression changes at various stages of differentiation, or by counting nodules and measuring osteocalcin secretion two weeks after treatment with glucocorticoid was initiated. Treatment of these cultures with 100-500 μM PFA for two weeks completely abolished mineralization of nodules and reduced osteocalcin secretion by 90% in a dose-dependent manner. Levels of mRNA for Bone Morphogenetic Protein-6 (BMP-6, an important mediator of osteoblast mineralization in these cultures) were reduced 55% after 12 h of PFA treatment. Other markers of the osteoblast phenotype (osteopontin mRNA, alkaline phosphatase activity) were also dose-dependently inhibited by these doses of PFA. Using a mature osteoblast-like cell line, we determined that PFA has no effect on either proliferation of these cultures or responses to calcitropic hormones (PTH, 1,25 vitamin D_3). We conclude that PFA may exert deleterious effects on bone by inhibiting osteoblast differentiation and bone mineralization. As AIDS patients receive more effective, life-prolonging treatment, this potential side-effect of PFA may become apparent. (This work was supported by the GA VA Research Center on AIDS and HIV Infections.)

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Viral thymidine kinase is essential for the spread of herpes simplex virus (HSV) induced zosteriform lesions *in vivo*.

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In mice following an i.p. infection, HSV-2 distributes to multiple sites, particularly the peripheral and central nervous systems. Subsequent replication within the CNS produces a lethal encephalitis. In contrast, infection of the skin surface with HSV-2 results in a discrete local lesion, migration of virus to sensory neuronal ganglia innervating the infection site, and subsequent spread of the virus to adjacent areas of the same dermatome to produce a strip of ulcerative lesions (the zosteriform band). In both types of infection, virus replication within neuronal tissue is important for pathogenesis, and this replication is dependent on the activity of virally-encoded thymidine kinase (TK). Thus, TK^{-ve} HSV-2 is not lethal following a systemic infection, and does not induce a zosteriform lesion following skin innoculation (although it does induce a primary lesion at the infection site). We show that the phenotype of a TK^{-ve} virus can be reproduced *in vivo* by the therapeutic administration of specific inhibitors of viral TK. In particular, Ro 32-1520 and Ro 32-4397 prevented HSV-2 induced lethality in a systemic infection model, and reduced zoster lesion development in a model of dermal infection. TK-dependent HSV replication following viral reactivation in neuronal tissue is thought to preceed the emergence of virus at mucosal surfaces, suggesting that inhibitors of viral TK will have therapeutic potential in the control of recurrent HSV infection in the clinic.

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The Relationship of Basal Cell Layer Drug Concentration (C*) to the Antiviral Efficacy: A Rigorous Evaluation of the C* Concept for Predicting the Entire Range of Topical Efficacy of Acyclovir Formulations in the Treatment of Cutaneous HSV-1 Infections in Hairless Mice. Samir C. Mehta, Mohsen I. Afouna, Abdel-Halim Ghanem, William I. Higuchi, Earl R. Kern[a] and Hamed El-Shattawy[b]. Dept. of Pharmaceutics and Pharmaceutical Chemistry, Univ. of Utah, Salt Lake City, UT 84112; [a]Dept. of Pediatrics, Univ. of Alabama at Birmingham, AL 35294; [b]Dept. of Pharmaceutics, Univ. of Al-Azhar, Cairo, Egypt.

For the past few years, our laboratory has been involved in the development of a novel approach for predicting topical *in vivo* efficacy based on the estimation of skin target site free drug concentration (C*) from *in vitro* flux data. We have used acyclovir (ACV) as a model drug in the treatment of cutaneous HSV-1 infections in hairless mice. The goal of this study was to rigorously evaluate the applicability of this approach over the entire range of topical efficacy (i.e., from 0% to 100%). We employed a variety of ACV formulations differing in solvent compositions, enhancers, and excipients (and therefore in their efficacies) to achieve this goal. C* values were estimated from the *in vitro* flux data obtained in an *in vivo-in vitro* experimental design that closely approximated the *in vivo* treatment protocol. For the *in vivo* antiviral efficacy studies, a finite dose of ACV formulation was applied twice a day, beginning the day after virus inoculation, for four days. The lesions were scored on the fifth day and the efficacies were calculated as described earlier (Gonsho *et al., Int. J. Pharm.*, 65, 183-194 (1990)). Our results indicate that, for a variety of formulations over a wide range of efficacies, the predictions based on C* are in good agreement with the observed *in vivo* efficacies. These findings strongly demonstrate the predictive value of C* over the entire range of topical efficacy, thereby further strengthening its potential for use in practical situations. The findings also indicate that although the excipients in a formulation may alter the rate and extent of available drug at the target site, in these cases, they do not have any effect on the *in vivo* potency of the drug. Supported by a Grant-in-Aid from TheraTech, Inc. and by NIH Grant AI 20161.

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The Antiviral Efficacy of Topical ZOVIRAX® Cream or VECTAVIR® Cream in HSV-Infected Mice.

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This animal model was used to compare the efficacy of ZOVIRAX® Cream (acyclovir, ACV) to VECTAVIR® Cream (penciclovir) in three single-blinded studies. The third study included placebo-treated controls and mice given an oral dose of ACV equivalent to the topical dose (oral controls). SKH-1 or HRS/J mice were inoculated on the snout with HSV-1 (SC-16, wild-type HSV) and treated topically b.i.d. with either ZOVIRAX® Cream or VECTAVIR® Cream. The orofacial HSV mouse model is designed to mimic an episode of human HSV labialis recurrence. After inoculation mice show visible lesions usually about four days after inoculation. Since many people experience prodrome 8 to 24 hours before recurrent lesions are visible, the topical treatments were started on day three post-inoculation and the mice were scored daily for lesion severity. Area-under-the-curve values were calculated for each mouse and were compared using a two-sided T-test. In all experiments, treatment with ZOVIRAX® Cream was significantly better than VECTAVIR® Cream, untreated controls, oral controls, and placebo-treated controls (All P values were < 0.001) in preventing lesion severity. Placebo-treated controls were not significantly different from untreated controls (P=0.29). Oral controls were not significantly different from untreated controls (P=0.16) proving that the activity seen with ZOVIRAX® Cream is not due to ingestion of topically applied material. The effects of VECTAVIR® Cream were not statistically different from untreated (P=0.64, 0.71, and 0.63) or placebo-treated controls (P=0.13). In conclusion, these experiments show that ZOVIRAX® Cream is superior to VECTAVIR® Cream in preventing HSV lesion development in this model when treatment is initiated three days post-inoculation.