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ANTI-RETROVIRUS ACTIVITY AND PHARMACOKINETICS IN MICE OF BIS(POC)-PMPA, THE BIS(ISOPROPYLOXYCARBONYL-OXYMETHYL) ORAL PRODRUG OF PMPA.

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The potent anti-retrovirus agent (*R*)-9-(2-phosphonylmethoxypropyl)-adenine (PMPA) has recently entered Phase I/II trials in HIV-infected patients. The low oral bioavailability of PMPA, which requires daily intravenous infusion, could be by-passed by the oral administration of a lipophilic ester prodrug. From a large number of newly synthesized ester prodrugs of PMPA, the bis(isopropyloxycarbonyloxymethyl) ester [bis(POC)-PMPA] was selected for further evaluation in animal models. Following its oral administration in dogs or mice, bis(POC)-PMPA was readily cleaved to free PMPA; no intact bis(POC)-PMPA could be detected in plasma. The oral bioavailability of PMPA when administered as such or as bis(POC)-PMPA prodrug was estimated as <3% and 25~30%, respectively. Oral bis(POC)-PMPA proved markedly effective in suppressing murine sarcoma virus (MSV)-induced tumor formation in SCID mice, its efficacy being comparable to that of subcutaneous PMPA, given at equimolar doses. For instance, in mice receiving 10-day treatment of oral bis(POC)-PMPA at a daily dose of 200, 100 or 50 mg of PMPA equivalent per kg, mean days of MSV-induced tumor appearance were: 13.5, 12.6 and 8.6 days, respectively (untreated control mice: 5.5 days), which is comparable to the data obtained with subcutaneous PMPA at daily doses of 200, 100 or 50 mg/kg (mean days of tumor appearance: 16.2, 13.8, and 9.8 days, respectively). In a five-day repeat dose toxicity study in dogs, oral bis(POC)-PMPA, given at a dose of 60 mg of PMPA equivalent per kg per day, was associated with marginal toxicity. Our animal data are in strong support of the clinical development of bis(POC)-PMPA as an orally bioavailable and non-toxic prodrug for PMPA.

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Line Probe Assay (LiPA) for HIV-1 RT: Prevalence of Drug-Selected Resistant Isolates in HIV-1 Infected VA Patients. Stuyver, L.,[1*] Wyseur, A.,[1] Rombout, A.,[1] Rimland, D.,[2] Rossau, R.,[1] and Schinazi, R.F.[2] Innogenetics NV, Gent, Belgium;[1] and Georgia VA Res. Ctr. for AIDS and HIV Infections (RCAHI), VAMC/Emory University, Decatur, GA, USA.[2]

The aim of this study was to determine the genetic variability and the prevalence of different genetic motifs in those regions of the HIV-1 RT gene that have been described to confer resistance to antiretroviral agents. A prospective study of HIV-1 infected patients from the VA Medical Center was initiated in 1994. To date, 314 plasma samples have been collected from 86 different patients treated with multiple FDA-approved antiretroviral compounds. We designed a diagnostic tool, the Line Probe Assay (LiPA), based on the reverse hybridization principle, allowing the detection of single nucleotide differences in subregions of the HIV-1 RT gene. A total of 59 specific probes were optimized, covering wild-type and mutant sequence motifs for the codons 41, 69, 70, 74, 75, 151, 184, 215, and 219. The HIV-1 RT region between codon 29 and 220 was amplified with biotinylated PCR primers.

A total of 284 (91%) of the samples were found PCR positive. PCR-negativity occasionally emerged as a consequence of initial successful therapy, but follow-up samples (two or three samples later) were subsequently PCR positive in all patients. The 284 samples were tested against 59 specific probes yielding a total of 16,756 determinations. In those cases where LiPA interpretation was uncertain, PCR fragments were sequenced for confirmation. A great variety of wild type and mutant motifs was detected in this patient population. As expected with 3TC, the M184V change dominated over complex intermediate forms (several polymorphisms, including 184I) within 3 months of treatment. Intermediate polymorphisms were rare for other drug-related codon changes. 184V did not prevent further development of AZT-resistance mutations at codon 41 and 215. One sample contained the 151M multi-drug resistance mutation. Viral load increase coincided with the appearance of genetic resistance patterns. (DR and RS are supported by the VA RCAHI).

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Phosphorylation of Zidovudine when Dosed in an *In Vitro* Pharmacodynamic System that Simulates *In Vivo* Pharmacokinetics. F. Hamzeh, J. Lee, M. Huber, A. Shahkolahi, H. Farzadegan, G. Wang and, P. Lietman. Division of Clinical Pharmacology, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

We have previously described an *in vitro* pharmacodynamic system in which drugs can be dosed and eliminated to mimic human pharmacokinetics. We studied the relationship between zidovudine's pharmacokinetics and its intracellular phosphorylation kinetics in CEM cells. Zidovudin's phosphorylation kinetics may partially determine how best to dose this drug. We investigated four dosing regimens: once daily, twice daily, three times daily, and as a continuous infusion. Zidovudine concentrations in the circulating medium and intracellular Z, ZMP, ZDP, and ZTP were measured at various time points. The table shows the AUC/24 hours for extracellular Z and the intracellular AUC/24 hours of ZMP, ZDP, ZTP.

Dosing regimen	AUC/24h (μmole.h/L)	AUC/24h (pmole.h/10^6 cells)		
	Z	ZMP	ZDP	ZTP
Q24H	19.2	768.9	23.4	41.5
Q12H	26.4	1374.5	38.9	47.4
Q8H	23.7	1005.1	32.8	46.1
Cont.Infusion	9.0	320.1	33.6	42.3

There was a clear dose dependent accumulation of the ZMP which correlates with the extracellular Z concentrations. Z C_{max}s were 8, 4.3, 2.6 and 0.37 μM for the q24, q12, q8 hours and continuous dosing, respectively. The corresponding ZMP C_{max}s were 140, 120, 80 and 5 pmole.h/10^6 cells. However, the intracellular ZDP and ZTP peaked at 2-3 hours after dosing and stayed between 2 to 3 pmole.h/10^6 cells for more than 12 hours at which time the ZMP concentration reached 5 to 10 pmole.h/10^6. ZTP AUCs per 24 hours, which are presumably better predictors of efficacy than extracellular Z AUCs, were similar for the four dosing regimens. The data suggest that ZTP concentrations reached a plateau very quickly after dosing. The data also suggest that increasing the dose of Z will not influence ZTP concentrations because of the large ZMP pool.

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Stavudine (d4T) and Didanosine (ddI) Combination Therapy in HIV-Infected Subjects: Analysis of Antiviral Effect and Safety in a Randomized Double-Blind Study. R POLLARD*, D PETERSON, D HARDY, L PEDNEAULT, V RUTKIEWICZ, J POTTAGE, R MURPHY, J GATHE, G BEALL, J SKOVRONSKI, A CROSS, L DUNKLE. U. of TX Galveston, U. of TX Dallas, U. of CA Los Angeles, Bristol-Myers Squibb Pharmaceut. Research Institute, Rush Medical College, Chicago, Northwestern U., Chicago, Houston Clinical Research Network.

A total of 86 treatment-naive HIV-infected subjects with CD4 cell counts of 200-500/mm^3 started therapy with one of the following d4T+ddI combinations administered BID: 40 mg+200 mg, 20 mg+200 mg, 40 mg+100 mg, 20 mg+100 mg, 10 mg+100 mg (doses were adjusted for weight <60 kg). Baseline characteristics were: mean age 33 yrs, mean CD4 count 343 cells/mm^3, and mean HIV RNA levels 4.1 $\log_{10}$. Median duration of therapy was 51 weeks, ≥52 weeks for 37 subjects. Sixty-six subjects were evaluable for virologic response (i.e., ≥ 1000 HIV RNA copies/ml at baseline). Sustained mean decreases of 1.1 to 1.5 $\log_{10}$ in HIV RNA and mean increases of 70 to 92 CD4 cells/mm^3 were observed at week 28 across dose groups. Follow-up at 52 weeks was available for ≥ 40% evaluable subjects; mean 1.2 $\log_{10}$ decrease in HIV RNA and mean 97 CD4 cells/mm^3 above baseline were seen. Subjects receiving the full recommended dose of at least one of the two drugs maintained significantly better increase in CD4 cell counts (146 vs 45/mm^3) and decrease in HIV RNA (1.3 vs 1.0 $\log_{10}$) at week 52, than subjects receiving ≤ half the recommended doses. Adverse events were few and not dose-related.

In conclusion, these findings suggest that d4T+ddI combination therapy has potent antiviral effect, and that suppression of viral load is sustained for at least one year. The full recommended dose of each drug is well tolerated in combination and appears to provide superior antiviral effect.