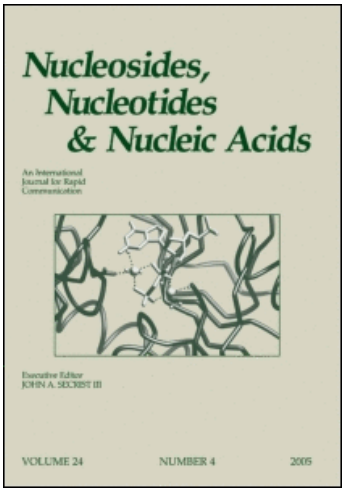


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# *In vivo* Antiretroviral Efficacy of Oral *bis*(POM)-PMEA, the *bis*(Pivaloyloxymethyl)prodrug of 9-(2-Phosphonylmethoxyethyl) adenine (PMEA)

L. Naesens[a]; J. Neyts[a]; J. Balzarini[a]; N. Bischofberger[b]; E. De Clercq[a]
[a] Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium [b] Gilead Sciences, Inc., 353 Lakeside Drive, Foster City, California

**To cite this Article** Naesens, L. , Neyts, J. , Balzarini, J. , Bischofberger, N. and De Clercq, E.(1995) '*In vivo* Antiretroviral Efficacy of Oral *bis*(POM)-PMEA, the *bis*(Pivaloyloxymethyl)prodrug of 9-(2-Phosphonylmethoxyethyl) adenine (PMEA)', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 767 — 770
**To link to this Article: DOI:** 10.1080/15257779508012468
**URL:** http://dx.doi.org/10.1080/15257779508012468

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 767-770 (1995)

# *IN VIVO* ANTIRETROVIRAL EFFICACY OF ORAL BIS(POM)-PMEA, THE BIS(PIVALOYLOXYMETHYL)PRODRUG OF 9-(2-PHOSPHONYLMETHOXYETHYL)ADENINE (PMEA)

L. Naesens[1]*, J. Neyts[1], J. Balzarini[1], N. Bischofberger[2] and E. De Clercq[1]

[1]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium;*

[2]*Gilead Sciences, Inc., 353 Lakeside Drive, Foster City, California 94404*



**ABSTRACT:** The bis-pivaloyloxymethyl(POM)- and diphenyl-ester prodrugs of the broad spectrum antiviral agent 9-(2-phosphonylmethoxyethyl)adenine (PMEA) have been evaluated *in vivo* for antiviral efficacy upon oral administration in severe combined immune deficiency (SCID) mice infected with Moloney murine sarcoma virus (MSV). Oral bis(POM)-PMEA proved highly efficient in delaying MSV-induced tumor formation and associated death, its effect being equal to that of subcutaneous PMEA at an equimolar dose. Compared to bis(POM)-PMEA, oral diphenyl-PMEA had lower antiviral efficacy, whereas PMEA as such was poorly effective when administered orally. Our studies indicate that bis(POM)-PMEA must have a favorable oral bioavailability and justify its clinical investigation as an oral prodrug of PMEA in the treatment of HIV infections.

## INTRODUCTION

The broad-spectrum antiviral agent 9-(2-phosphonylmethoxyethyl)adenine (PMEA) is currently being explored in Phase I/II trials for its efficacy against HIV (human immunodeficiency virus) infections[1]. Due to its limited oral bioavailability, PMEA needs to be injected, which is a drawback for long-term clinical use. Oral therapy may be accomplished by the administration of ester prodrugs of PMEA, in which the anionic phosphonate moiety is linked to a lipophilic side chain. Studies in monkeys have shown that the bis-pivaloyloxymethyl(POM)-ester of PMEA has an oral bioavailability of about 25%, compared to less than 1% as reported for

PMEA as such[2,3]. We have now demonstrated that bis(POM)-PMEA is indeed highly efficient as an antiretroviral drug *in vivo* when administered orally, its efficacy being equal to that of systemic PMEA.

## MATERIALS AND METHODS

***MSV infection of SCID mice.*** Three-week-old SCID (severe combined immune deficiency) mice (weighing about 12 g) were infected with Moloney murine sarcoma virus (MSV), by intramuscular injection in the left hind leg. Appearance of MSV-induced tumors and associated death of the mice was followed daily. On days 5, 7 and 10 post infection (p.i.), the tumor size was measured with a caliper.

***Antiviral drug treatment.*** Drug treatment was performed twice daily from day 0 until day 4. Compounds were compared at equimolar doses, i.e., at 100 and 50 mg/kg/day for PMEA; 184 and 92 mg/kg/day for bis(POM)-PMEA; and 156 and 78 mg/kg/day for diphenyl-PMEA. PMEA was dissolved in water in the case of peroral (p.o.) treatment, or in phosphate-buffered saline in the case of subcutaneous (s.c.) injection. Fresh preparations of bis(POM)-PMEA and diphenyl-PMEA were prepared daily. These oral formulations of bis(POM)-PMEA and diphenyl-PMEA contained less than 10% of mono-ester and 0% of PMEA, as shown by HPLC analysis. The administration volume was 0.5 ml and 0.2 ml for p.o. and s.c. administration, respectively.



## RESULTS AND DISCUSSION

Intramuscular inoculation of Moloney murine sarcoma virus (MSV) in SCID mice resulted in the formation of massive tumors at the site of virus inoculation within 5 days p.i., and eventually caused death of the mice within 14 days p.i. Subcutaneous (s.c.) treatment with PMEA at a dose of 20, 50 or 100 mg/kg/day effected a marked and dose-dependent delay in MSV-induced tumor formation and associated death (Table 1). By contrast, oral PMEA at a dose of 50 or 100 mg/kg/day was found to have relatively low efficacy. However, oral therapy with the bis(pivaloyloxymethyl) ester prodrug of PMEA [bis(POM)-PMEA] afforded a marked antiviral response. Oral bis(POM)-PMEA at a dose of 184 mg/kg/day proved equally efficient as s.c. PMEA given at an equimolar dose of 100 mg/kg/day, the mean day of tumor appearance being 10.5 days and 10.6 days, respectively, as compared to 5.0 days for untreated control mice. Compared to bis(POM)-PMEA, diphenyl-PMEA had weaker antiviral potency upon oral administration.

TABLE 1. Antiviral efficacy of PMEA, bis(POM)-PMEA and diphenyl-PMEA upon oral administration to MSV-infected SCID mice[a].

| Compound[b] Route | Dose (mg/kg/day)[c] | Number of mice | Mean day of tumor appearance (± SD) | Mean day of death (± SD) |
|---|---|---|---|---|
| PMEA p.o. | 100 | 5 | 7.0 ± 0.0 | 17.6 ± 1.7 |
| | 50 | 5 | 7.0 ± 0.0 | 15.4 ± 2.3 |
| PMEA s.c. | 100 | 5 | 10.6 ± 0.9 | 19.4 ± 0.9 |
| | 50 | 5 | 8.8 ± 0.8 | 16.2 ± 1.1 |
| | 20 | 5 | 7.8 ± 0.8 | 17.0 ± 0.7 |
| bis(POM)-PMEA p.o. | 184 | 4 | 10.5 ± 1.0 | 18.3 ± 0.5 |
| | 92 | 5 | 8.4 ± 1.1 | 15.8 ± 1.5 |
| diphenyl-PMEA p.o. | 156 | 5 | 7.2 ± 0.4 | 14.2 ± 1.3 |
| | 78 | 5 | 7.6 ± 0.6 | 14.2 ± 1.3 |
| Control | 0 | 9 | 5.0 ± 1.5 | 13.9 ± 1.0 |

[a]Data given are the results of one representative experiment. Similar results were obtained in a second experiment.
[b]For oral administration, bis(POM)-PMEA and diphenyl-PMEA were dissolved in 10% ethanol in water. PMEA was dissolved in water (p.o. administration) or in buffered saline (s.c. administration).
[c]Compounds were administered twice daily at the daily doses indicated, from day 0 until day 4.



Pharmacokinetic studies in rats have revealed for PMEA, diphenyl-PMEA and bis(POM)-PMEA an oral bioavailability of 7.8%, 11.1% and 10.6%, respectively[4]. In monkeys, PMEA was reported to have far lower oral bioavailability than its bis(POM)-prodrug (1% and 25%, respectively)[3,4]. A marked difference in oral bioavailability is also suggested by our antiviral data in MSV-infected SCID mice.

The remarkable anti-MSV effect of oral bis(POM)-PMEA is also apparent from Fig. 1. At days 5 and 7, tumors of mice receiving oral treatment with bis(POM)-PMEA were considerably smaller than in control animals. However, after cessation of treatment, tumors in drug-treated animals finally became as large as in control animals. Thus, it appears that continuous antiretroviral treatment is necessary to keep the virus under control. Indeed, resurgence of viral symptoms when treatment is stopped is a general observation in AIDS patients treated with antiretroviral nucleoside analogues such as AZT, ddC or ddI.

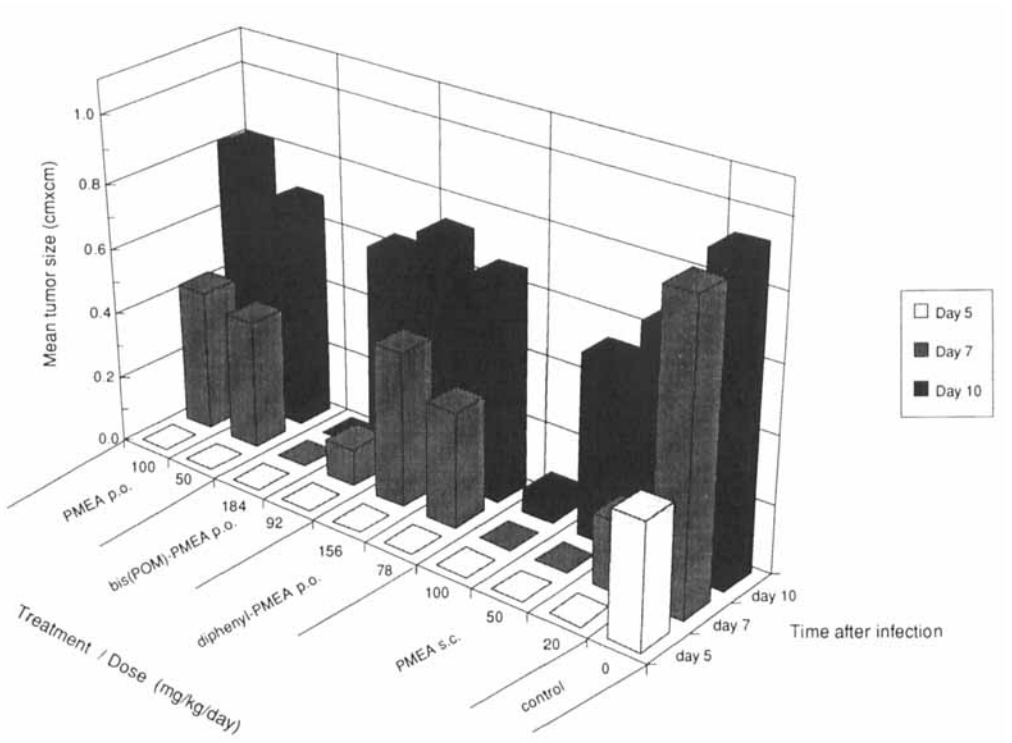


FIG. 1. Inhibitory effect of oral PMEA, bis(POM)-PMEA and diphenyl-PMEA against MSV-infected tumor formation in MSV-infected SCID mice.



In conclusion, we have demonstrated that bis(POM)-PMEA exhibits marked antiviral efficacy when administered orally. The low oral bioavailability of PMEA, which is a drawback for long-term clinical use, can thus be overcome by the administration of its oral prodrug bis(POM)-PMEA. Clinical studies in HIV-infected individuals should now be performed to investigate the pharmacokinetics, antiviral efficacy and toxicology of bis(POM)-PMEA in humans.

## REFERENCES

1. Naesens, L., Balzarini, J. and De Clercq, E. *Rev. Med. Virol.*, in press (1994).
2. Cundy, K. C., Fishback, J. A., Shaw, J.-P., Lee, M. L., Soike, K. F., Visor, G. C. and Lee, W. *Pharm. Res.*, **11**: 839–843 (1994).
3. Balzarini, J., Naesens, L., Slachmuylders, J., Niphuis, H., Rosenberg, I., Holy, A., Schellekens, H. and De Clercq, E. *AIDS*, **5**: 21–28 (1991).
4. Starrett, J. E., Tortolani, D. R., Russell, J., Hitchcock, M., Whiterock, V., Martin, J. C. and Mansuri, M. M. *J. Med. Chem.*, **37**: 1857–1864 (1994).