Journal of Chromatography, 414 (1987) 429–433
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3470

Note

High-performance liquid chromatographic method for the determination of 9-(1,3-dihydroxy-2-propoxymethyl)guanine in human plasma

JEAN-PIERRE SOMMADOSSI* and RAE BEVAN

Department of Pharmacology and Comprehensive Cancer Center, Division of Clinical Pharmacology, University of Alabama at Birmingham, Birmingham, AL 35294 (U.S.A.)

(First received September 1st, 1986; revised manuscript received October 15th, 1986)

9-(1,3-Dihydroxy-2-propoxymethyl)guanine (DHPG, BW B759U, 2′-NDG, BIOLF-62) (Fig. 1) is an acyclic nucleoside analogue of the guanine base which has shown promising therapeutic effects in the treatment of cytomegalovirus infections [1, 2]. A few methodologies which include radioimmunoassay (RIA) [3] and enzyme-linked immunosorbent assay [4] have been reported for the quantification of this antiviral drug in biological fluids. Although these immunological techniques appear, in general, more sensitive and useful in the analysis of a large number of samples than a high-performance liquid chromatographic (HPLC) method, they have also significant disadvantages. These latter include the length of time (approximately 24 h) to obtain final quantitative results, the large number of steps in their procedure, and the need to develop antiserum [3] and/or monoclonal antibodies [4].

In the present paper, we describe a simple sensitive and highly selective HPLC method which, by its greater speed, appears of particular interest for therapeutic drug monitoring and/or pharmacokinetic studies of this antiviral drug.

EXPERIMENTAL

Chemicals and reagents

[^{3}H]DHPG and cold DHPG were generously supplied by Dr. W.C. Buhles [Syntex (U.S.A), Palo Alto, CA, U.S.A.]. Heptane sulfonic acid was purchased from Sigma (St. Louis, MO, U.S.A.). All aqueous solutions were prepared with double-distilled water and other chemicals were of HPLC and/or reagent-grade quality.

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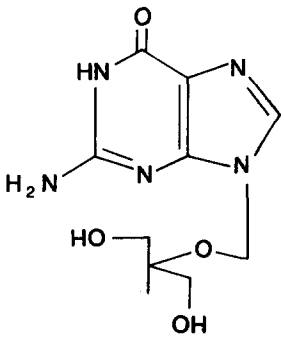


Fig. 1. Chemical structure of DHPG.

The heptane sulfonic acid–phosphate buffer used for the mobile phase was a mixture of 5 m*M* heptane sulfonic acid and 20 m*M* potassium phosphate buffer. This solution was adjusted to pH 2.6 with concentrated hydrochloric acid and filtered through a 0.22-μm membrane filter (Millipore, Bedford, MA, U.S.A.). Stock solutions of DHPG were prepared at 1 mg/ml in 0.1 *M* hydrochloric acid and working concentrations achieved by serial dilutions in water.

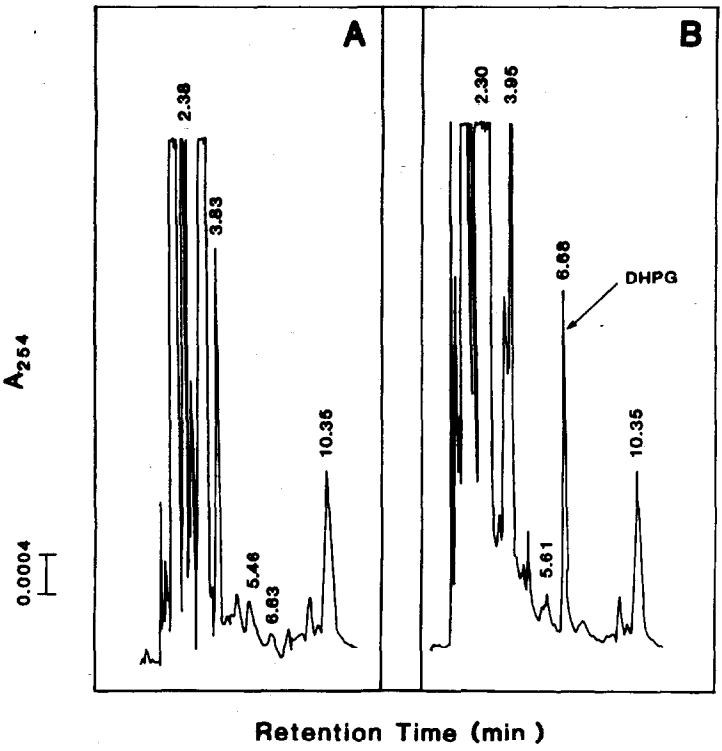
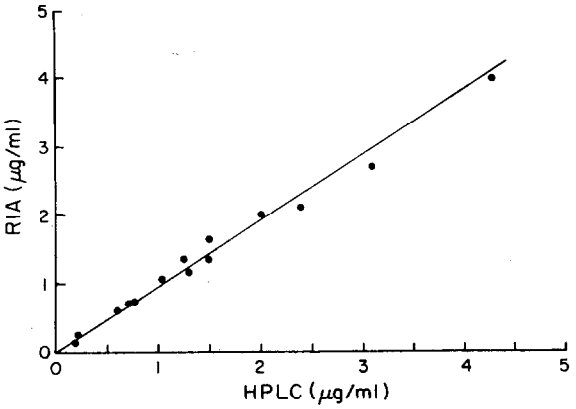


Fig. 2. Chromatograms of (A) deproteinized plasma control and (B) deproteinized patient's plasma containing 0.5 μg/ml DHPG (retention time 6.68 min).



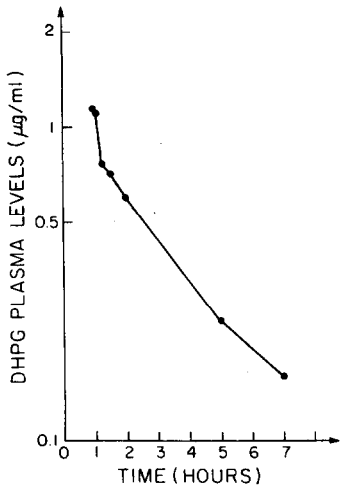


Fig. 3. Methods comparison plot of DHPG plasma concentration determined by HPLC and RIA. Slope = 1.0; $r = 0.999$ and $n = 14$.

Fig. 4. Plasma levels of DHPG in a patient who received 48 mg of drug by 1-h i.v. infusion.

Apparatus and chromatographic conditions

The liquid chromatograph (Hewlett-Packard 1084B) was equipped with an automatic injector, fixed-wavelength UV detector (254 nm) and a chromatographic terminal (Hewlett-Packard 79850 ALC). All analyses were performed using a 5-μm, RP-18 column, 250 mm×4 mm I.D. (IBM Instruments, Poughkeepsie, NY, U.S.A.) as the stationary phase. Elution was carried out isocratically at a flow-rate of 2 ml/min with a mixture of 5 m*M* heptane sulfonic acid and 20 m*M* potassium phosphate buffer (pH 2.6)–acetonitrile (97.5:2.5, v/v).

Procedure

Blood samples from patients receiving DHPG were drawn by venipuncture and collected in heparinized tubes. These were immediately centrifuged at 3000–4000 *g* for 10 min at 4°C. Plasma was separated and stored at −20°C until analysis.

Aliquots of plasma (200 μl) were diluted with 40 μl of water and deproteinized by addition of 20 μl of 50% trichloroacetic acid in 1.5-ml plastic microcentrifuge tubes. These samples were vortexed for 30 s and then centrifuged at 15 000 *g* in an Eppendorf Model 5412 microcentrifuge for 1 min. Portions of the supernatant (150 μl) were neutralized with 1 *M* potassium hydroxide (19 μl) and 100-μl aliquots were analyzed by the HLPC method described above.

Standard calibration curves were obtained after analysis of plasma samples to which increasing quantities of DHPG from 0.1 to 5 μg/ml were added. Curves showed linear correlations between concentration and peak height, yielding, for DHPG, a correlation coefficient (r) of 0.9989 with a typical regression equation of $y = 13.29x + 0.27$.

Kinetic studies

In a preliminary pharmacokinetic study, a dose of 1 mg/kg DHPG (total dose 48 mg) was administered over 1 h by intravenous (i.v.) infusion to a patient with acquired immunodeficiency syndrome (AIDS) and evidence of cytomegalovirus infection. Plasma concentrations were analyzed as described above and pharmacokinetic parameters were calculated using a non-compartmental analysis based on statistical moment theory [5].

RESULTS

Typical chromatograms obtained from patient's plasma before and after administration of DHPG are shown in Fig. 2. The chromatographic peak of the analyzed drug was well resolved with a retention time of 6.68 min. It should be noted that the HPLC column was equilibrated each morning with a mixture of heptane sulfonic acid and phosphate buffer (pH 2.6)–acetonitrile (90:10, v/v) for 30 min to 1 h at a flow-rate of 2 ml/min. No interferences from endogenous plasma substances were detected and co-administration of prednisone, nystatin and Bactrim was not found to interfere with the analysis. An ion-pair system was found necessary in order to remove endogenous background detected at the retention time of DHPG when a simple phosphate mobile phase was performed.

The absolute recovery of this technique was calculated using [^{3}H]DHPG as a tracer and recovery values ranging between 95 and 98% were obtained.

The detection limit for routine assays was 100 ng/ml when 200 μl plasma were used. Intra-assay precision was determined by replicate analysis ($n=10$) of control plasma to which 500 ng/ml DHPG was added. The obtained concentration was 486.2 ± 9.3 ng/ml (mean $\pm$ S.D.) which yielded a coefficient of variation of 1.9% at this concentration.

Quantification of DHPG by this novel HPLC method was compared with a RIA technique recently reported by Nerenberg et al. [3]. For this purpose, clinical plasma samples ranging from 0.1 to 4 μg/ml DHPG were analyzed by both techniques. Linear regression analysis of the data measured by HPLC versus RIA (Fig. 3) for fourteen plasma samples resulted in a straight line with a slope of 1 and a correlation coefficient of 0.999, indicating excellent agreement between the two techniques.

Using this HPLC techniques, we studied the decrease with time of DHPG plasma levels in a AIDS patient who received 48 mg of drug by a 1-h i.v. infusion. The plasma concentration–time curve is illustrated in Fig. 4. A preliminary pharmacokinetic approach using a non-compartmental analysis yielded an apparent elimination half-life of 2.65 h with a total plasma clearance of 13.02 l/h.

CONCLUSION

DHPG is a novel antiviral agent that has shown potential efficacy against cytomegalovirus infections in AIDS patients [1, 2]. Immunological techniques have been recently reported for the quantification of this drug in biological samples [3, 4]; however, extensive periods of time, approximately 24 h, are required before

obtaining final results. This is particularly inconvenient when a quick answer is needed during drug therapy. Until now, use of an HPLC method to quantify DHPG in plasma has been mainly limited by a lack of sensitivity [3, 6]. The technique described in the present report, by an increased sensitivity of more than five-fold compared to previous techniques [3, 6] eliminates this major disadvantage.

In conclusion, this novel HPLC method is particularly useful when a sensitive and rapid determination of DHPG in plasma is needed.

ACKNOWLEDGEMENTS

We thank Dr. Richard Whitley for providing the clinical specimens and Dr. Clinton Nerenberg, Syntex (U.S.A.), Palo Alto, CA, U.S.A. for supplying RIA reagents and helpful advice concerning the assay. This study was supported by Grant CA 13148 and by Syntex Research Co.

REFERENCES

1 Collaborative DHPG Treatment Study Group, N. Engl. J. Med., 314 (1986) 801.
2 D.H. Shepp, P.S. Dandliker, P. de Miranda, T.C. Burnette, D.M. Cederberg, L.E. Kirk and J.D. Meyers, Ann. Intern. Med., 103 (1985) 368.
3 C. Nerenberg, S. McClung, J. Martin, M. Fass, J. LaFargue and S. Kushinsky, Pharm. Res., 3 (1986) 112.
4 S.M. Tadepalli, R.P. Quinn and D.R. Averett, Antimicrob. Agents Chemother., 29 (1986) 93.
5 M. Gibaldi and D. Perrier, Pharmacokinetics, Marcel Dekker, New York, 2nd ed., 1982, p. 409.
6 P. de Miranda and D.A. Page, 24th Intersci. Conf., Antimicrob. Agents Chemother., 24 (1984) 1189.