Pergamon

0731-7085(94)E0029-Z

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 9, pp. 1205–1207, 1994

0731-7085/94 $7.00 + 0.00

Short Communication

Stability of ganciclovir in blood samples

R. BOULIEU*†‡ and N. BLEYZAC†‡

†*Institut des Sciences Pharmaceutiques et Biologiques (ISPB), Laboratoire de Pharmacie Clinique, 8 Avenue Rockefeller, 69373 Lyon Cedex 08, France*
‡*Hôpital Cardiovasculaire et Pneumologique, Service Pharmaceutique, B.P. Lyon, Montchat, 69394 Lyon Cedex 03, France*

Keywords: *Ganciclovir; stability; blood.*

Introduction

Ganciclovir is a nucleoside analogue with antiviral activity against human herpes virus [1]. This drug is widely used for treating cytomegalovirus (CMV) infections in organ-transplant patients [2].

Ganciclovir being eliminated almost exclusively by renal excretion [3], the dosage will be adjusted for patients with renal impairment. Because of potent toxicity, frequency of recurrence and occurrence of clinical resistant strain of CMV to ganciclovir therapy, careful monitoring of ganciclovir concentration is needed in patients with renal dysfunction. Some high-performance liquid chromatographic (HPLC) methods for the determination of ganciclovir in plasma samples have been reported in the literature [4–8]. However, stability of ganciclovir in biological samples during transport and storage have not been studied. Therefore, the stability of ganciclovir in blood and plasma samples in various conditions of storage has been investigated.

Materials and Methods

Reagents

Ganciclovir was purchased from Syntex (Paris, France), potassium dihydrogenphosphate, orthophosphoric acid and perchloric acid were obtained from Merck (Nogent-sur-Marne, France).

Chromatographic instrumentation and conditions

Ganciclovir was analysed using a high-performance liquid chromatographic method described previously [8]. The chromatographic apparatus consisted of a Model 420 pump equipped with a model 430 variable wavelength detector (all from Kontron, St Quentin les Yvelines, France), a Model 712 WISP sample processor (Waters, St Quentin les Yvelines, France) and a D2000 Chromato-integrator (Merck). The stationary phase was Hypersil ODS, 3 μm (Touzart et Matignon, Vitry/Seine, France) and the mobile phase consisted of 0.02 mol l^{-1} potassium dihydrogenphosphate at a pH of 5.25. The flow rate was 1.5 ml min^{-1} and the detection was performed at 254 nm.

Sample treatment

Plasma samples (500 μl) were deproteinized with 50 μl of 35% perchloric acid. After centrifugation at 2000*g* for 15 min at 4°C, the supernatants were removed and 20 μl aliquots were injected on to the column.

Procedure for stability studies

Stability in blood samples. Blood samples were spiked with ganciclovir at a concentration of 7.5 mg l^{-1} and separated in two aliquots, one was stored at room temperature (20°C) and the other was placed on an ice-bath (0°C). For each sample, 2 ml aliquots were drawn at

* Author to whom correspondence should be addressed.

the following intervals: 0, 15, 30, 60 and 120 min. At these times, blood samples were immediately centrifuged at 4000 rpm for 15 min at 4°C. Plasma was decanted and deproteinized without delay for HPLC analysis. This assay was replicated six times.

Stability in plasma samples. The stability of ganciclovir was assayed in spiked plasma stored at room temperature or in an ice bath.

Ganciclovir was added to human blank plasma at a concentration of 5.0 mg l^{-1}. Separate aliquots were stored at room temperature or in an ice bath. At the following intervals: 0.15, 30, 60 and 120 min, 500 µl of plasma were drawn and immediately deproteinized for analysis.

Ganciclovir stability was also evaluated in plasma samples stored at −20°C and −80°C for 4 weeks. Spiked plasma with ganciclovir at a concentration of 5.0 mg l^{-1} were divided into separate aliquots stored at −20°C or at −80°C and analyzed within 0, 1, 2, 3 and 4 weeks of storage.

Statistical analysis

The non parametric Mann–Whitney test was used for statistical comparison with a level of significance of $P < 0.05$.

Results

Table 1 shows the result of the stability study of ganciclovir in blood samples. When the blood sample was left only for 15 min at room temperature before centrifugation, ganciclovir

Table 1
Stability of ganciclovir in blood samples

Time of storage (min)	Temperature (°C)	Ganciclovir concentration in plasma (mg l^{-1}, mean ± SD, $n = 6$)	Percentage of initial concentration (%, mean ± SD)	Mann–Whitney test (comparison to values determined at initial time)
0	0	7.70 ± 0.30	100	—
	20	6.45 ± 0.20	100	—
15	0	7.95 ± 0.20	103 ± 3	NS
	20	5.10 ± 0.10	79 ± 1	$P < 0.01$
30	0	7.50 ± 0.10	98 ± 1	NS
	20	5.05 ± 0.20	78 ± 3	$P < 0.01$
60	0	7.30 ± 0.10	95 ± 2	NS
	20	4.75 ± 0.10	74 ± 1	$P < 0.01$
120	0	6.65 ± 0.15	86 ± 2	$P < 0.01$
	20	4.65 ± 0.20	72 ± 3	$P < 0.01$

Table 2
Stability of ganciclovir in plasma samples

Time of storage	Temperature (°C)	Ganciclovir concentration in plasma (mg l^{-1}, mean ± SD, $n = 6$)	Percentage of initial concentration (%, mean ± SD)	Mann–Whitney test (comparison to values determined at initial time)
0 min	0	4.90 ± 0.20	100	—
	20	4.90 ± 0.20	100	—
15 min	0	4.90 ± 0.20	100 ± 4	NS
	20	4.95 ± 0.25	101 ± 5	NS
30 min	0	4.90 ± 0.25	100 ± 5	NS
	20	4.90 ± 0.10	100 ± 3	NS
60 min	0	4.80 ± 0.20	98 ± 4	NS
	20	4.85 ± 0.15	99 ± 3	NS
120 min	0	4.85 ± 0.20	99 ± 4	NS
	20	4.90 ± 0.15	100 ± 3	NS
0 week	−20	4.85 ± 0.10	100	—
	−80	4.85 ± 0.10	100	—
1 week	−20	4.80 ± 0.10	99 ± 3	NS
	−80	4.90 ± 0.10	100 ± 3	NS
2 weeks	−20	4.90 ± 0.10	101 ± 3	NS
	−80	4.85 ± 0.15	101 ± 3	NS
3 weeks	−20	4.90 ± 0.15	101 ± 3	NS
	−80	4.95 ± 0.10	102 ± 3	NS
4 weeks	−20	4.85 ± 0.10	100 ± 1	NS
	−80	4.90 ± 0.10	101 ± 2	NS

concentration decreased significantly from 6.45 to 5.10 ($P < 0.01$) resulting in an average loss of 21% of the initial concentration. After 2 h of storage at room temperature, ganciclovir concentration represents an average of 72% of the initial value. When the blood sample was stored in an ice bath, no significant decrease in ganciclovir concentration was observed.

The stability of ganciclovir in plasma samples stored in various conditions is shown in Table 2. No significant decrease in ganciclovir concentration occurred in plasma samples left for 2 h at room temperature or in an ice bath. Storage of ganciclovir at −20°C and −80°C did not result in significant changes in plasma concentrations over a 4-week period of storage.

Discussion

The experiment performed on blood samples shows that a significant fall in the concentration of ganciclovir occurred in whole blood left for only 15 min at room temperature between sampling and centrifugation. Furthermore, the initial plasma concentration found when the blood sample was left at room temperature was significantly lower ($P < 0.01$ Mann–Whitney test) than those obtained when the blood was kept in an ice bath. These results suggest that ganciclovir was unstable in plasma samples left in contact with red blood cells at room temperature. The significant fall observed in ganciclovir concentration may be explained by the rapid permeation of the drug into erythrocytes followed by a metabolic conversion that can occur in the cells. This fall did not occur when whole blood was kept at low temperature (0°C).

The transport of purine bases and nucleosides across human erythrocytes has been investigated [9–11]. Purine compounds cross the cell membrane in both directions by a single transport system [10]. The rate of uptake being less at lower temperature [12]. More recently, Mahony *et al.* [13] have reported that ganciclovir permeates human erythrocyte membrane essentially using nucleobase carriers.

Stability studies of ganciclovir in plasma samples showed that ganciclovir was stable in plasma left for 2 h at room temperature or in an ice bath. Furthermore, plasma samples can be stored for up to 4 weeks at −20°C before analysis.

The present study shows the necessity of observing rigorous conditions during collection, transport and treatment of blood samples to prevent ganciclovir permeation across erythrocyte membrane and metabolic conversion in the cells. Thus, in order to achieve an accurate determination of ganciclovir concentrations, the blood must be placed on ice for transport and centrifuged immediately at low temperature. The plasma samples can be stored at −20°C or −80°C over 4 weeks until HPLC analysis without any modification in the ganciclovir concentrations.

References

[1] A.K. Field, M.E. Davies, C. Dewitt, H.C. Perry, R. Liou, J. Germershansen, J.D. Karkas, W.T. Ashton, D.B.R. Johnston and R.L. Tolman, *Proc. Nat. Acad. Sci.* **80**, 4139–4143 (1983).
[2] D. Faulds and H.R.C. Heel, *Drugs* **39**, 597–638 (1990).
[3] O.L. Laskin, D.M. Cederberg, J. Mills, L.J. Eron and D. Mildvan, *Am. J. Med.* **83**, 201–207 (1987).
[4] C. Fletcher, R. Sawchuk, B. Chinnock, P. de Miranda and H.H. Balfour, *Clin. Pharmacol. Ther.* **40**, 281–286 (1986).
[5] J.P. Sommadossi and R. Bevan, *J. Chromatogr.* **414**, 429–433 (1987).
[6] E.H.H. Wiltink, P. Stekkinger, J.A.C. Brakenhoff and S.A. Danner, *Pharmaceut. Weekbl. Sci. Ed.* **9**, 261–264 (1987).
[7] M.A. Hedaya and R.J. Sawchut, *Pharmaceut. Res.* **7**, 1113–1118 (1990).
[8] R. Boulieu, N. Bleyzac and S. Ferry, *J. Chromatogr.* **567**, 480–484 (1991).
[9] R. Whittam, *J. Physiol.* **154**, 614–623 (1960).
[10] M.M. Müller and G. Falkner, *Adv. Exp. Med. Biol.* **76**, 131–138 (1977).
[11] R. Boulieu, C. Bory, P. Baltassat and C. Gonnet, *J. Liq. Chromatogr.* **7**, 1013–1021 (1984).
[12] B.A. Domin, W.B. Mahony and T.P. Zimmerman, *J. Biol. Chem.* **263**, 9276–9283 (1988).
[13] W.B. Mahony, B.A. Domin and T.P. Zimmerman, *Biochem. Pharmacol.* **41**, 269–271 (1991).

[Received for review 2 December 1993; revised manuscript received 17 December 1993]