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Short communication

High-performance liquid chromatographic determination of ganciclovir nucleotides in human myocardial tissue

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Abstract

A method for the analysis of ganciclovir nucleotides in myocardial tissues was developed. The antiviral effect of ganciclovir is attributed to intracellular ganciclovir nucleotides. The procedure is based on perchloric acid deproteinization and enzymatic hydrolysis of the ganciclovir nucleotides to ganciclovir. Then, the parent drug was analyzed on a Hypersil ODS column using potassium dihydrogenphosphate buffer as mobile phase. The mean analytical recovery of ganciclovir from myocardial tissue was 101 ± 2% and the detection limit was 2 pmol. The sample treatment procedure described is simple and presents a suitable analytical tool for the investigation of the ganciclovir nucleotides pool in tissues.

1. Introduction

Ganciclovir or 9-(1,3-dihydroxy-2-propoxymethyl) guanine is an antiviral drug used in immunocompromised patients with cytomegalovirus (CMV) infections [1]. The antiviral effect of ganciclovir results from its conversion to the triphosphate form which inhibits viral DNA synthesis [2–4]. The initial phosphorylation step to ganciclovir monophosphate appears to be the rate-limiting step and is catalyzed by cellular enzymes induced by CMV. As a consequence, intracellular concentrations of ganciclovir triphosphate in CMV-infected cells are higher than those in non-infected cells [3,5].

No data are available on the cellular concentrations of ganciclovir nucleotides *in vivo*. Moreover the lack of standards of nucleotides of ganciclovir represents a major drawback in the development of analytical methods. Therefore, we have developed a sample treatment procedure using perchloric acid deproteinization and enzymatic conversion prior to HPLC analysis for the determination of ganciclovir nucleotides in tissue samples. The method was applied to the analysis of ganciclovir and its nucleotides in heart tissue specimens from heart-transplant patients under ganciclovir therapy.

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2. Experimental

2.1. Reagents

Ganciclovir was purchased from Syntex (Paris, France). Potassium dihydrogenphosphate, orthophosphoric acid, perchloric acid and sodium hydroxyde were obtained from Merck (Nogent-sur-Marne, France). Alkaline phophatase, type VII-NT (10 000 glycine units /mg of protein) and guanosine triphosphate were from Sigma (St. Quentin Fallavier, France).

2.2. Chromatographic analysis

Ganciclovir was analysed using a high-performance liquid chromatographic method described previously [6]. The chromatrographic system consisted of a Hypersil ODS 3 μm, 150 × 4.6 mm I.D. column. Potassium dihydrogenphosphate (pH 3.5, 0.02 *M*) was used as the mobile phase. The flow-rate was 1.5 ml/min and detection was performed at 254 nm.

2.3. Sample collection and storage

Myocardial tissue samples were collected as part of graft rejection monitoring. Tissue samples were immediately frozen in liquid nitrogen to preserve the nucleotide pool and stored at −80°C until analysis. Simultaneously, blood samples of 5 ml were collected in heparinized tubes and centrifuged without delay at 2000 *g* for 15 min at low temperature. Plasma was stored at −20°C until analysis.

2.4. Sample treatment procedure

To myocardial tissue 0.6 *M* perchloric acid (700 μl/10 mg, v/w, precooled in ice water) was added and the sample was homogenized for 2.5 min at 6000 rpm in a stirpack homogenizer (Bioblock scientific, France). After centrifugation at 2000 *g* for 15 min at 4°C, the supernatants were adjusted to pH 7–8 with sodium hydroxide. A 200-μl volume of the supernatant was removed for ganciclovir analysis. An other 200-μl aliquot of supernatant was incubated with 10 μl of alkaline phosphatase for 30 min at 37°C. This procedure hydrolyses the mono-, di- and triphosphate nucleosides of ganciclovir to ganciclovir. Then, both the treated and the non-treated supernatant were evaporated at room temperature with a RC 1022 centrifugal evaporator (Jouan, St. Nazaire, France). The residues were reconstituted in 30 μl of mobile phase and 20-μl aliquots were injected onto the column. Plasma samples were deproteinized with perchloric acid and analysed according to the previously published method [6].

3. Results and discussion

Fig. 1 shows a chromatogram of blank myocardial tissue. Typical chromatograms of a myocardial sample (a) before and (b) after enzymatic hydrolysis from a heart transplant patient receiving 5 mg/kg/24 h of ganciclovir are shown in Fig. 2.

Due to the lack of standard ganciclovir nucleotides, the enzymatic conversion of nucleotide into nucleoside by the alkaline phosphatase was evaluated using guanosine triphosphate (GTP) as nucleotide standard. Under the conditions used, 96% of GTP was hydrolyzed to guanosine.

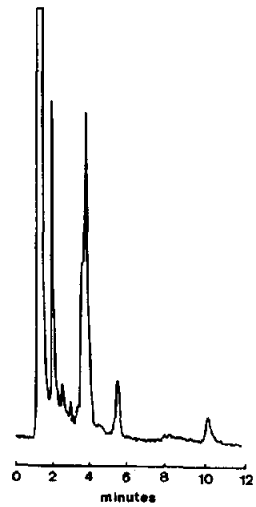

Fig. 1. Chromatogram of a blank myocardial tissue. Chromatographic conditions are described in the Experimental section. Injection volume: 20 μl.

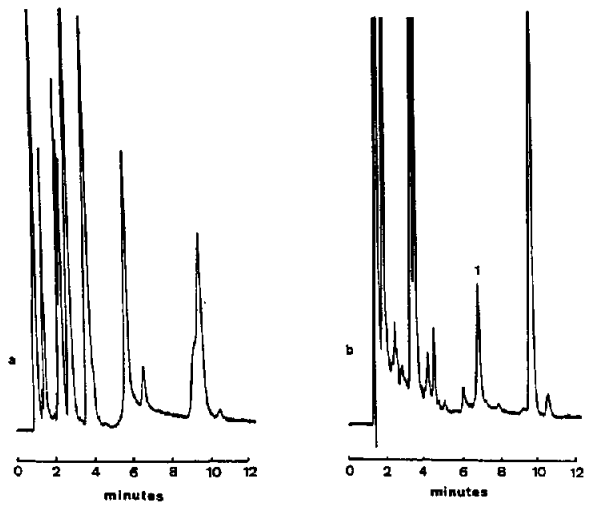

Fig. 2. Chromatograms of a myocardial tissue (a) before, and (b) after enzymatic hydrolysis from a heart transplant patient receiving 5.0 mg/kg/24 h of ganciclovir. Chromatographic conditions are described in the Experimental section. Injection volume 20 μl. Peak 1: ganciclovir.

The mean analytical recovery of ganciclovir from myocardial tissue determined by adding known concentrations of standard to the tissue specimens before sample processing was 101 ± 2% (mean ± S.D., $n = 5$). The relationship between the concentration and the peak height of ganciclovir was linear up to 40.0 μmol/l in the PCA extract, which corresponds to 2.8 nmol/mg of tissue. The minimum detectable amount, defined as a signal-to-noise ratio of 4 was found to be 2 pmol for ganciclovir; using the sample treatment procedure described this corresponds to 0.9 pmol/mg of tissue. The intra- and inter-assay coefficients of variation in myocardial tissue were 1.0% and 4.4% respectively with a deviation from the theoretical value of 1.0% ($n = 5$) at a concentration of 35 pmol/mg of tissue.

Ganciclovir nucleotide concentration in myocardial tissue and ganciclovir concentration in plasma were determined in five heart transplant patients treated with ganciclovir at a dosage of 2.5 mg/kg/12 h or 5.0 mg/kg/24 h for severe CMV infection (Table 1). In four patients, biopsy was performed four to five hours after the infusion and in one patient, tissue sampling was carried out after death.

As shown in Table 1, ganciclovir nucleotides were recovered from myocardial tissue of three patients. The concentrations found were higher in patients with histological signs of CMV myocarditis suggesting that ganciclovir is preferentially phosphorylated in CMV infected cells than in non-infected cells. These results are in agreement with the *in vitro* studies reported previously [3,5]. This study also shows that no ganciclovir was recovered from myocardial tissue.

In conclusion, the sample treatment procedure described for the analysis of ganciclovir nucleotides in tissue samples is simple and presents a suitable method for the investigation of the

Table 1
Concentration of ganciclovir in plasma and concentration of nucleotides of ganciclovir in myocardial tissue.

Patients	Dosage	Sampling time after infusion (h)	Ganciclovir concentration in plasma (μmol/l)	Ganciclovir nucleotides concentration in myocardial tissue (pmol/mg of tissue)
1	2.5 mg/kg/12 h	4	9.0	ND
2	5.0 mg/kg/24 h	4	21.6	3.6
3	5.0 mg/kg/24 h	4	13.0	ND
4[b]	5.0 mg/kg/24 h	5	9.4	47.1
5[b]	5.0 mg/kg/24 h	–[a]	–	42.6

ND: not detectable.
[a] Post-mortem tissue samples.
[b] Patients with CMV myocarditis.

intracellular ganciclovir nucleotide pool and the method should be useful for the diagnosis of CMV-myocarditis.

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