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Determination of prostaglandin E_1 in plasma with picogram per millilitre sensitivity by double antibody extraction and column switching high-performance liquid chromatography

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Abstract: Prostaglandin E_1 (PGE_1) is a vaso- and arteriodilator that is used for the experimental treatment of a variety of vascular diseases. A high-performance liquid chromatography (HPLC) method was developed for the purpose of quantifying PGE_1 in the plasma of patients undergoing constant infusion therapy. Plasma (0.5 ml) was extracted with a double antibody precipitation technique and the PGE_1-immunoprecipitate was isolated and washed by repeated resuspension and centrifugation. PGE_1 was recovered from the precipitate by extraction with acetonitrile and derivatized with panacyl bromide for determination on a heteromodel column switching HPLC system with fluorescence detection. The content of PGE_1 in immunoextracts was determined by HPLC peak height comparison against a standard curve of PGE_1 peak height vs amount derivatized. The result was corrected for the plasma extraction efficiency (determined with radiolabelled PGE_1) to obtain the plasma concentration. Standard curves were linear from 25 to 400 pg ($r > 0.99$) and the y-intercepts were not significantly different from zero ($p < 0.05$). The immunoextraction recovery from six human donors was 63.5 $\pm$ 2.0% (S.D., $n = 18$). The quantification limit of the method was 50 pg ml^{-1} (signal-to-noise ratio 3:1), at which the estimated assay relative standard deviation was 18%. The utility of the method for the measurement of PGE_1 plasma levels during constant intravenous infusion was demonstrated in a dog study.

Keywords: *Prostaglandin E_1; plasma; HPLC column switching; immunoprecipitation; double antibody extraction.*

Introduction

Prostaglandin E_1 (11α,15α-dihydroxy-9-oxoprost-13-enoic acid; PGE_1; U-10,136; alprostadil; the active ingredient in Prostin VR sterile solution, The Upjohn Company) is a natural product present, or readily biosynthesized, in many mammalian tissues [1]. Among its many pharmacological activities, PGE_1 is a potent vaso- and arteriodilator [2]. It has been approved for treatment of infants with congenital heart defects [3, 4] and

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has been used experimentally to treat peripheral vascular disease [5], glomerulonephritis [6] and collagen disease [7]. It is currently under development by the Upjohn Company to treat acute respiratory distress syndrome (ARDS). Typically, PGE_1 is infused into a central vein at a rate of 5 to 50 ng kg^{-1} min^{-1}. Pharmacokinetic calculations [8], assuming a pulmonary extraction ratio in man of 0.9 [9], predict that the steady state plasma concentration of PGE_1 resulting from these infusions is in the low picogram per millilitre range, but may be several fold higher in disease states accompanied by pulmonary dysfunction or shunting, as may occur in ARDS [10] or liver cirrhosis [11, 12]. Because of the potential for variability between subjects in the steady state plasma concentration of PGE_1 during infusion, it is of interest to attempt correlations of the efficacy of PGE_1 therapy with the circulating PGE_1 concentration.

Several methods have been devised to quantify PGE_1 in plasma at picogram per millilitre concentrations, including gas chromatography–mass spectrometry (GC–MS) [13–16] and radioimmunoassay (RIA) [17, 18]. Reversed-phase high-performance liquid chromatography (HPLC) methods with picogram range detection limits have been described for solution standards of PGE_1 [19–21], but none have been reported to be useful for quantification at low picogram per millilitre levels in physiological fluids. Besides problems associated with trace level derivatization, a major problem has been to obtain sufficiently purified extracts of plasma for HPLC analysis. HPLC methods do not have the selective detection capability of GC–MS methods and so must rely to a greater extent on the selectivity of the plasma cleanup procedure and chromatography system to achieve specificity.

The present report describes an HPLC method for PGE_1 in plasma with low picogram per millilitre sensitivity. Specificity was achieved by combining a plasma immunoextraction technique with a heteromodal column switching HPLC system [22]. The performance specifications of the method were evaluated and its utility was demonstrated with an infusion study in dogs. The quantification limit and linear dynamic range of the method should make it useful for the measurement of circulating PGE_1 levels in humans during constant infusion therapy.

Experimental

Chemicals and reagents

PGE_1 and panacyl bromide [*p*-(9-anthroyloxy)phenacyl bromide] were supplied by Pharmaceutical Research and Development, The Upjohn Company (Kalamazoo, MI). [5,6-^{3}H]PGE_1 (specific activity 55.8 Ci $mmol^{-1}$) was obtained from New England Nuclear (Boston, MA) and was purified before use to 99% radiochemical purity by thin layer chromatography (toluene–methylene chloride–methanol–methyl ethyl ketone–acetic acid, 20:20:5:5:1, v/v). Rabbit anti-PGE serum which was cross reactive with PGE_1 and PGE_2 [23] was elicited against 9-deoxy-9-methylene-$PGF_{2\alpha}$, a stable isosteric mimic of PGE_2, and was supplied by F.A. Fitzpatrick and M.A. Wynalda (Lipids Research, The Upjohn Company). Goat anti-rabbit γ-globulin serum was provided by D.P. Kane (Upjohn Diagnostics). Extraction and chromatography solvents were UV or HPLC grade from Burdick and Jackson Labs (Muskegon, MI). Working solutions of PGE_1 in acetonitrile (25 and 2.5 ng ml^{-1}) were prepared fresh daily from a stock solution (74 μg ml^{-1}) by serial dilution. The stock solution was prepared in acetonitrile and was stable for at least one month at −20°C. All solution transfers were made with polypropylene tipped pipettors and all solutions were stored in polypropylene tubes.

Plasma collection and fortification with PGE_1

Plasma for PGE_1 measurement and for recovery experiments was collected from human volunteers by arm venipuncture and from dogs by jugular venipuncture. Blood was drawn into all plastic Monovette® 10 ml EDTA tubes (Sarstadt, Inc., Princeton, NJ). The tubes were placed on ice and centrifuged within 30 min of collection at 1500 ***g*** for 30 min at 4°C. The plasma was stored at −20°C until use. Human plasma for assay precision and accuracy measurements was obtained in 0.6 l lots with sodium citrate as the anticoagulant from Plasma Alliance (Knoxville, TN). Plasma was fortified with PGE_1 by evaporating aliquots of PGE_1 working solutions under nitrogen in 12 × 75 mm polypropylene tubes and reconstituting in 0.5 ml of plasma by vortexing for 1 min.

Prostaglandin immunoprecipitation

Frozen plasma samples (0.5 ml in a 12 × 75 mm polypropylene tube) were thawed in a water bath at room temperature and rabbit anti-PGE_1 serum (0.060 ml of a 1:10 dilution in 0.1 M Tris–HCl buffer, pH 7.8, containing 0.1 mg ml^{-1} of thimerosal) was added. The tubes were vortexed for 30 s and then shaken on a horizontal shaker (Eberbach Corp., Ann Arbor, MI) at 3 cycles s^{-1} for 30 min. Goat anti-rabbit serum (0.030 ml undiluted) was added and the tubes were vortexed for 30 s and incubated overnight at 4°C. Samples were then vortexed for 1 min and centrifuged for 30 min at 1500 ***g***. The supernatant was carefully decanted by inversion and the lip of the tube touched to a paper towel to remove the last drop. The pellet was resuspended in distilled water (1.0 ml) by vortexing for 1 min and centrifuged as before. The supernatant was decanted and the wash cycle repeated once more. After decanting the wash, 1.0 ml of acetonitrile was added and the tubes vortexed for 1 min, shaken at 6 cycles s^{-1} on a horizontal shaker for 10 min, and centrifuged for 10 min at 1500 ***g***. The acetonitrile extract (0.90 ml) was then transferred to another 12 × 75 mm tube using a polypropylene tipped pipette.

Prostaglandin derivatization

Acetonitrile extracts of the immunoprecipitates were evaporated to dryness at 40°C under a stream of nitrogen. The residues were reconstituted in 0.25 ml of panacyl bromide solution (25 μg in tetrahydrofuran–acetonitrile, 1:4, v/v) by vortexing for 30 s, transferred to polypropylene microvials (0.5 ml volume) and derivatized as described previously [22]. Then the samples were evaporated at 40°C under nitrogen and reconstituted in 0.25 ml of isooctane–ethylene dichloride–propan-2-ol (70:30:1, v/v). The vials were capped with an aluminium seal containing a 0.01 in thick Teflon disc (SCI/SPEC, Randallstown, MD), sonicated for 10 min in an immersion bath (Branson Cleaning Equipment, Shelton, CT) and placed in the autosampler of the HPLC system. The injection volume was 0.20 ml.

High-performance liquid chromatography

The analytical chromatography system (a modification of the apparatus described previously, Fig. 1) [22] consisted of two isocratic systems (1 and 2) linked serially via a 2.2 ml sampling loop which permitted the direct injection of system 1 eluant into system 2. Mobile phase 1 was hexane–methylene chloride–propan-2-ol (70:30:1, v/v). Mobile phase 2 was hexane–methylene chloride–tetrahydrofuran–propan-2-ol (55:10:35:1, v/v). Samples (200 μl) were injected with an ISS-100 autosampler (Perkin Elmer, Norwalk, CT) equipped with a constant temperature injection tray cooled to 4°C by a circulating refrigeration bath (Endocal RTE-8, Neslab, Newington, NH). The guard column (5 cm

× 4.6 mm i.d.) was slurry packed at 6000 psi in propan-2-ol with Zorbax BP-CN (cyanopropylsilane bonded phase, 7–8 μm, log No. 18341-140, DuPont Co., Wilmington, DE). Analytical column 1 was a Zorbax CN, 15 cm × 4.6 mm i.d., and column 2 was a Zorbax SIL, 25 cm × 4.6 mm i.d. (both packing materials are spherical particles, 6 μm diameter, from DuPont Co.).

The system control program provided for an initial vent to waste (Position I, 0–2.9 min) from the guard column to remove most of the unreacted panacyl bromide (Fig. 1). An eluant fraction taken from the guard column was routed to column 1 (Position II, 2.9–3.7 min) and then the guard column eluant was routed back to waste (Position I, 3.7–9.0 min). An eluant fraction was taken from column 1 by collecting the desired fraction in a 2.2 ml loop (Position III, 9.0–10.1 min). This fraction was injected onto column 2 and the PGE_1 panacyl ester was quantified by fluorescence detection (Position I, 10.1–34.0 min) with excitation at 375 nm and emission at 470 nm [22]. Mobile phase flow rates were 2 ml min^{-1} for all columns from 0 to 10.1 min and then 1 ml min^{-1} for column 2, 3 ml min^{-1} for column 1, and 2 ml min^{-1} for the guard column from 10.1 to 34 min. At 34 min, the cycle was repeated.

The switching times listed above are approximate and were determined daily as follows. A [^{3}H]PGE_1 panacyl ester standard equivalent to 440 pg of PGE_1 (8000 dpm) was injected and the guard column eluant was manually collected in 0.2 ml fractions, evaporated to dryness under nitrogen and reconstituted in liquid scintillation cocktail for radioactivity quantitation. The guard column switching times were set so that greater than 90% of the PGE_1 peak was transferred to column 1. The column 1 switching times were then established by injecting unlabelled PGE_1 panacyl ester standard (equivalent to 4 ng of PGE_1) and recording the baseline-to-baseline column 1 retention times with detector 1. The variation in switching times between batches of mobile phases was typically <0.3 min for both the guard column and column 1.

Plasma PGE_1 quantification method

Standards were prepared from working solutions in duplicate at 25, 50, 100, 200 and 400 pg, and individually derivatized as described above. HPLC data were analysed by unweighted linear regression of PGE_1 peak height versus picograms derivatized. The assay result was divided by a correction factor (plasma volume × fractional extraction recovery) to obtain the plasma concentration. The extraction recovery was determined for individual plasma samples by fortifying 0.5 ml with 70 pg of [^{3}H]PGE_1 and extracting in parallel with the samples for HPLC analysis. The extraction recovery was calculated as dpm recovered divided by dpm added.

Dog pharmacokinetic study

One purebred beagle dog (12 kg) was infused, via the right jugular vein using a surgically fitted cannula extending to the right atrium, with a PGE_1 saline solution at 80 ng kg^{-1} min^{-1} for 40 min and 320 ng kg^{-1} min^{-1} for another 40 min. Another beagle was infused via the right cephalic vein using a butterfly infusion needle at 320 ng kg^{-1} min^{-1} for 40 min. Dosing solutions were infused at a rate of 3.0 ml h^{-1} using a syringe drive pump. Blood samples were collected by left jugular venipuncture at timepoints before, during, and after the infusion as described above except that the first 4 ml of blood drawn from each venipuncture was discarded.

Results

High-performance liquid chromatography

The heteromodal column switching HPLC system previously described for E class prostaglandins [22] was optimized in the present work for PGE_1 quantification. An attempt was made to delete column 1 (Fig. 1) from the system and route a fraction of the guard column eluant directly onto column 2 for quantification, but a plasma component that coeluted with PGE_1 from the guard column (cyano bonded phase) eluted much later from column 2 (silica) and interfered with subsequent injections. Reinserting column 1, an analytical cyano bonded phase column, and instituting a wash cycle for the column after PGE_1 had been transferred to column 2 eliminated the interference. Reckoned from the time of the initial injection, the PGE_1 panacyl ester typically eluted in 3.7 min from the guard column, 10 min from column 1, and 32 min from column 2. The analytical run/recycle time was 34 min.

Structurally related eicosenoids, including PGE_2, PGA_2, PGB_2, PGD_2, 13,14-dihydro-15-epi-PGE_1, 15-methyl-PGE_1,6-keto-$PGF_{1\alpha}$,T_xB_2, and C8, C11 and C15 positional epimers of PGE_1, had different retention times on either column 1 or 2 than PGE_1 and consequently did not interfere with chromatographic quantitation. Because of the high chromatographic specificity, an internal standard could not be identified. Precautions were therefore taken to minimize the injection volume variability and to control for changes in the chromatography retention times that could significantly affect the calculated PGE_1 peak height response factor. The autoinjector sample tray was refrigerated to reduce injection solvent evaporation and the injection conditions were

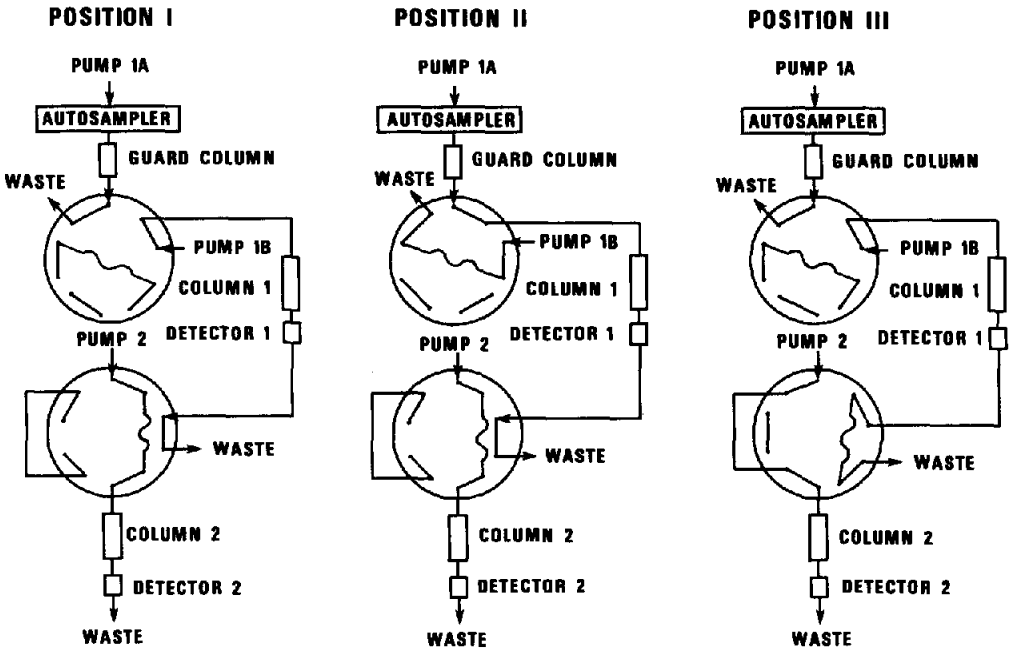

Figure 1
Schematic diagram of the HPLC system and switching valve configurations used for the analysis. See text for a description of the column switching program.

optimized so that the injection volume precision was less than 2% relative standard deviation (RSD) ($n = 7$). Changes in the composition of the HPLC mobile phase due to evaporation during overnight runs, which could have affected retention times and consequently peak transfer efficiencies during column switching, were minimized by enclosing the solvents in glass vessels under positive pressure [22]. The typical within-run RSD in the PGE_1 chromatography system retention time was 0.15% ($n = 31$) over a 19 h period. As a quality control check for each run, a PGE_1 panacyl ester solution standard was injected at the beginning and end of each set of samples. The peak heights of these standards were compared within the run as well as between runs to verify that the column switching times were set correctly and that the chromatography conditions were stable.

The linearity and reproducibility of the method were evaluated by comparing calibration curve results from four days. Five levels of PGE_1 from 25 to 400 pg were derivatized in duplicate. Correlation coefficients for plots of PGE_1 peak height versus picograms derivatized exceeded 0.99 and the residual errors were randomly distributed. The slopes of the calibration curves ranged from 0.64 to 0.74 mV pg^{-1} and the y-intercepts were not significantly different from zero ($p < 0.05$). The detection limit of the method was 13 pg, at which the signal-to-noise ratio was 3:1 and 10 pg equivalents of PGE_1 were injected on-column.

PGE_1 immunoextraction

PGE_1 was isolated from plasma by double antibody precipitation and centrifugation. The conditions for PGE_1 immunoprecipitation were established for 0.5 ml of human plasma fortified with 400 pg of [^{3}H]PGE_1 (8000 dpm). A preliminary experiment indicated that the recovery of radioactivity in centrifugation pellets reached a maximum after 10 min of centrifugation at 150 ***g***, and 10 min centrifugations were therefore used routinely. The concentration of the primary antiserum (Ab-1) and secondary antiserum (Ab-2) were varied in a matrix design experiment in which varying amounts of Ab-1 were added; the tubes were shaken for 15 min, and then varying amounts of Ab-2 were added. After 2 h of shaking at room temperature, the tubes were centrifuged and the supernatant was decanted and counted to determine the precipitation efficiency. Nearly maximal efficiency was achieved with a 1:100 dilution of Ab-1 and a 1:20 dilution of Ab-2. These dilutions were used in subsequent experiments and the incubation periods were lengthened to 30 min after adding Ab-1 and 16 h (overnight) at 4°C after adding Ab-2. The immunoprecipitation efficiency under these conditions was 78 ± 1% (S.D., $n = 6$) and was constant over a concentration range of 100–1000 pg ml^{-1}.

The precipitate was isolated from plasma components by repeated resuspension and centrifugation. The pellet from the first centrifugation step was resuspended by vortexing with 1.0 ml of water and then recentrifuged. Starting with [^{3}H]PGE_1 fortified human plasma and carrying through three wash cycles, 21.5 ± 1% of the radioactivity was lost in the supernatant after the first centrifugation step, 7.8 ± 0.7% after the second, 5.1 ± 0.4% after the third, and 1.4 ± 0.3% after the fourth (±S.D., $n = 6$). The pellet was then extracted by vortexing for 1 min and shaking for 10 min with 1.0 ml of acetonitrile. The precipitate was again sedimented by centrifugation and the acetonitrile extract was decanted and counted. It contained 58.1 ± 0.2% ($n = 3$) of the original sample radioactivity, or 90% of the radioactivity remaining in the tube after three washes.

To verify that the extraction recovery determined with radiotracer was unbiased by radiochemical impurities or PGE_1 degradation during the extraction process, extracts of these samples were also analysed by HPLC for PGE_1 content. The extraction recovery

by HPLC was 58.9 ± 2.5% (n = 3), in agreement with the radiotracer result and indicating that [^{3}H]PGE_1 could be used accurately to assess the extraction recovery.

In an experiment designed to determine the intersubject variability in extraction recovery, 10-ml blood samples were drawn into tubes containing EDTA by arm venipuncture from six volunteers (three males, three females; one male and one female had fasted, the others had eaten breakfast 2 h previously). Aliquots of plasma from each subject (2.0 ml) were fortified with 1.6 ng of PGE_1-^{3}H and frozen in 0.5 ml portions before further processing. After thawing, the fortified plasma from each subject was extracted in triplicate using two centrifugation washes and the acetonitrile extract was decanted and counted. There were no significant differences between the results of PGE_1 recovery experiments on the six subjects (one-way analysis of variance, p = 0.65). The intersubject mean recovery was 63.5% (R.S.D. = 3.1%, n = 18). Similarly, the intrasubject PGE_1 recovery was shown to be unaffected by mild or moderate hemolysis or by incubation of the blood at room temperature for 30 min or at 4°C for 60 min before centrifugation (p = 0.29). However, there was a significant difference (2.9%) between the extraction recovery from identical plasma samples on different days (p = 0.02).

PGE_1 quantification

No peak was detected with the retention time of PGE_1 in HPLC chromatograms of blank human or dog plasma immunoextracts so long as precautions were taken when drawing the blood. If collected in EDTA tubes through a catheter with a heparin lock or by venipuncture after discarding the first few millilitres of blood from each draw, the chromatographic baseline was free of interferences. However, a peak coeluting with PGE_1 and corresponding to approximately 50 pg ml^{-1} was detected in immunoextracts of the commercial human plasma and pooled dog plasma used for the assay validation (Fig. 2). The latter plasma was collected from several dogs by venipuncture without discarding the initial portion of each draw. Because the presence of the peak depended on the blood collection method and its size could not be diminished by repeated centrifugation washing of the immunoprecipitate, it was assumed that it represented endogenous PGE_1. The best estimate of the endogenous plasma concentration of PGE_1

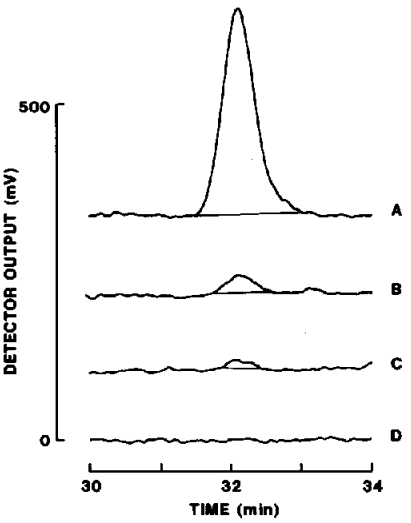

Figure 2
HPLC chromatograms of fortified dog plasma: (a) 500 pg ml^{-1} standard; (b) 50 pg ml^{-1} standard; and (c) plasma blank. Chromatogram (d) is for dog plasma collected under different conditions, showing the absence of a PGE_1 peak when the first 4 ml of blood from a venipuncture is discarded before collection of the sample.

in healthy adult humans is 3 pg ml^{-1} [18], but artificially high concentrations can be obtained if catheters are not used to draw the blood sample or if problems are encountered during the collection [24].

The content of PGE_1 in immunoextracts was determined by HPLC peak height analysis against a PGE_1 standard curve. The result was then divided by the extraction recovery and the plasma volume to obtain the plasma concentration. The extraction recovery and extract derivatization efficiency were shown to be constant over the concentration range of interest by analysing sets of human and dog plasma fortified with 50, 100, 200, 500 and 1000 pg ml^{-1} on three separate days. Plots of plasma standard PGE_1 peak height versus concentration were linear ($r > 0.99$) and had significantly positive y-intercepts corresponding to the endogenous PGE_1 concentration of 50–60 pg ml^{-1}. The interassay RSD of the PGE_1 peak heights were 18, 14, 9 and 7% for human plasma concentrations of 50, 100, 150 and 1050 pg ml^{-1} (endogenous plus fortified concentrations of PGE_1), respectively ($n = 6$). Using a standard curve and correcting for recovery losses, the intraassay accuracy for the determination of PGE_1 in human plasma samples fortified with 95 and 501 pg ml^{-1} was 97 and 108%, respectively, with assay results of 92 ± 9 pg ml^{-1} (R.S.D. = 10%, $n = 5$) and 540 ± 15 pg ml^{-1} (R.S.D. = 3%, $n = 5$) after subtraction of the endogenous PGE_1 concentration. The quantification limit of the assay was 50 pg ml^{-1} (signal-to-noise ratio 3:1).

Dog infusion study

One dog was infused via the jugular vein with a PGE_1 saline solution at 80 ng kg^{-1} min^{-1} for 40 min and then 320 ng kg^{-1} min^{-1} for another 40 min. Another dog was infused via the cephalic vein at 320 ng kg^{-1} min^{-1} for 40 min. This rate is approximately 10 times the infusion rate used in humans, but is justified for validation experiments in the dog because of the dog's higher cardiac output per kilogram body mass (see Discussion). As shown in Table 1, the predose levels of PGE_1 in both dogs were below the assay detection limit of 50 pg ml^{-1}. In the case of the dog with jugular infusion, plasma levels of PGE_1 were not detected at the 80 ng kg^{-1} min^{-1} infusion rate (not shown in Table 1), but reached an apparent plateau of approximately 130 pg ml^{-1} after 20 min at the 320 ng kg^{-1} min^{-1} infusion rate. The dog with cephalic infusion exhibited a maximum of 334 pg ml^{-1} after 30 min of infusion and the level decreased by 50% to 167 pg ml^{-1} after 40 min. The PGE_1 plasma concentration in both dogs was below the quantification limit 15 min after stopping the infusion. The recovery from the plasma of these dogs was approximately 65% and there was no significant difference

Table 1
Plasma concentrations of PGE_1 (pg ml^{-1}) following intravenous infusion in beagle dogs at 320 ng kg^{-1} min^{-1}*

Dog number	Time (min)†						
(infusion route)	−15	−5	20	30	40	55	70
1 (jugular)	ND,ND	ND,ND	120,128	137,134	—	ND,ND	ND,ND
2 (cephalic)	ND,ND	ND,ND	235,263	337,330	168,166	ND,ND	ND,ND

*Duplicate 0.5 ml samples of plasma were assayed at each timepoint. Both results are given. Assay results below the quantification limit of 50 pg ml^{-1} are marked ND, not detected.

†The infusion began at 0-time and was stopped at 40 min.

within dogs between samples taken at 15, 30 and 70 min. There was good agreement between the duplicate assay results on all specimens.

Discussion

The success of this analytical method depended upon achieving a high degree of specificity in both the plasma extraction and chromatography stages of the analysis. Although the HPLC system described here was specific for PGE_1 among structurally related eicosenoids, it was not specific enough for the direct quantification of PGE_1 in crude plasma extracts. When PGE_1 was extracted from plasma with octadecylsilane columns [25], the derivatized extract contained several components eluting in the vicinity of PGE_1 which interfered with quantification. In contrast, plasma extracts prepared with anti-PGE serum were free of interfering components so long as the complete column switching HPLC system was used. If the HPLC system was simplified by deleting column 1 there were unacceptable assay interferences. This finding may be significant for the HPLC determination of trace levels of other prostanoids. Even with a highly specific extraction method, it may be necessary to design greater specificity into the HPLC system than is present in most published HPLC methods for prostaglandins [19–21]. Nevertheless, the commercial availability of antisera against a variety of eicosenoids (including PGE) should facilitate the development of other sensitive HPLC quantitation methods.

There are relatively few reports in the literature on the use of antibodies to isolate plasma components for routine chromatographic determination. Gaskell *et al.* extracted steroids from plasma or serum with solid phase coupled antisera for determination by GC–MS [26–28], Krause *et al.* extracted iloprost, a stable prostacyclin analog, for GC–MS determination using a similar technique [29], and a double antibody precipitation method was used to isolate arbaprostil, a PGE_2 analog, for HPLC determination [30]. The arbaprostil immunoprecipitate was isolated by filtration. In the present example, the immunoprecipitate containing PGE_1 was isolated by repeated suspension and centrifugation. Because the immunoextraction steps depend on antibody binding, the accuracy of the analytical result is still potentially dependent on the uncertain influences of antibody cross reactivity and matrix effects, both of which can affect the accuracy of RIA results [18, 24]. Unlike the RIA, however, these effects can be minimized by using an excess of antibody. Under the conditions of this analysis, the extraction recovery of PGE_1 was independent of the PGE_1 concentration up to at least 1050 pg ml^{-1}, constant within and between individuals, and relatively insensitive to the plasma collection procedure. Furthermore, the method was designed so that the extraction recovery for each sample was determined in parallel with PGE_1 quantification, and adjustment of the analytical result could therefore be made for variable recovery if necessary.

The utility of the method for plasma PGE_1 determination during infusion treatment was demonstrated in a dog study. The plasma concentrations of PGE_1 achieved during infusion in the two dogs at a rate of 320 ng kg^{-1} min^{-1} ranged from 125 to 335 pg ml^{-1}, compared to undetectable levels both before the infusion and 15 min after stopping the infusion. These concentrations were similar to the concentrations predicted by pharmacokinetic calculations. Assuming for simplicity that the lung is the only site of PGE_1 elimination [9, 31], and assuming a pulmonary extraction ratio for PGE_1 of 0.9 in the dog [31], a cardiac output of 3 l min^{-1} [32], and a hematocrit of 0.45 (PGE_1 does not

distribute significantly into red blood cells), the predicted [8] steady state plasma concentration for a 320 ng kg^{-1} min^{-1} infusion rate is 300 pg ml^{-1}.

The same calculations for a 70 kg healthy man at a therapeutically relevant infusion rate of 30 ng kg^{-1} min^{-1} predict a steady state concentration of approximately 100 pg ml^{-1}. For a disease state in which the apparent pulmonary extraction ratio is reduced to 0.6, as reported for acute respiratory distress syndrome [33], the predicted steady state plasma concentration increases six times to 600 pg ml^{-1}. Thus, the analytical quantification limit and linear dynamic range of the method should be adequate for the measurement of PGE_1 in humans during infusion therapy.

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