6-Keto PGE₁: A Possible Metabolite of Prostacyclin Having Platelet Antiaggregatory Effects

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SUMMARY Hepatic metabolism of prostacyclin (PGI₂) results in the formation of several biologically inactive lipids and one stable product that has the same chromatographic and biological properties as authentic 6-keto PGE₁. Both prostaglandins, 6-keto PGE₁ and PGI₂, have comparable potency in their antiaggregatory and disaggregatory effects on platelets. They contract the superfused rat stomach strip but differ in their effects on the bovine coronary artery, which is contracted by 6-keto PGE₁ but relaxed by PGI₂. Further, 6-keto PGE₁ is considerably more stable than PGI₂. Thus, 6-keto PGE₁ could account for some of the prolonged effects occasionally seen with PGI₂. (Hypertension 2: 524-528, 1980)

KEY WORDS • prostacyclin • 6-keto PGF₁α • 6-keto PGE₁ • liver metabolism • platelets

ROSTACYCLIN (PGI₂), the principal product of arachidonic acid metabolism of blood vessels, is a potent inhibitor of platelet aggregation. It shares with prostaglandins of the E series the ability to lower blood pressure (BP) and relaxes vascular smooth muscle. PGI₂ escapes substantial metabolism on passage across the pulmonary vascular bed, a property required for a circulating hormone. Between 35% to 65% of infused prostacyclin was estimated to be inactivated on one circulation, presumably due to metabolism during transit through the liver, hind quarters, and kidney. As PGI₂ is unstable, having a half-life of approximately 3 minutes in aqueous solution, pH 7.6, 37ºC and in blood, the formation of a stable metabolite having equivalent biological potency would be of considerable importance. It has been shown that 6-keto PGE₁, a stable prostaglandin, has considerable platelet antiaggregatory potency and, like PGI₂, escapes pulmonary inactivation, reduces blood pressure, and decreases renovascular resistance. The present study suggests that 6-keto PGE₁ may arise during the course of metabolic transformation of PGI₂ by the liver.

Methods

Isolated Perfused Liver

Metabolism of PGI₂ was studied in the isolated perfused liver of the male New Zealand rabbit. After pentobarbital anesthesia, the liver was exposed by midline laparotomy. Following cannulation of the portal vein, the liver was flushed with Tyrode’s solution, removed from the animal, and placed in a thermostatically controlled chamber where it was perfused (10 ml/min with oxygenated (95% O₂, 5% CO₂) Tyrode’s solution (37ºC) using a Harvard peristaltic pump. Subsequent stabilization of the preparation, a 5-minute infusion of [9-³H]-PGI₂, diluted with authentic PGI₂ sodium salt (2 mg) to a specific activity of 1.8 µCi/mM, commenced. The venous effluent was collected during the PGI₂ infusion and thereafter for an additional 2 minutes. After recirculation, the final perfusate was collected in a container immersed in ice, acidified with 1 N HCl to pH 3.0, and extracted three times with equal volumes of ethyl acetate which were combined and evaporated to dryness.

Separation of Metabolites

The dry crude lipid extract was dissolved in chloroform/methanol/water mixture (86/14/1, v/v and passed through a Sephadex G-25 column (1 x 1 cm) which binds nonlipid contaminants. The lipid material was eluted with the same solvent and dried under nitrogen. Aliquots (200 µl) of the residue, dissolved in 1 ml acetone, were applied to thin-layer...
chromatographic plates (Brinkman), co-migrated with authentic standards, 6-keto PGF$_{10}$, 6-keto PGE$_{1}$, PGF$_{2\alpha}$, 6,15-diketo PGF$_{10}$, 6,15-diketo-13,14-dihydro PGF$_{10}$, PGE$_{2}$, pentanor PGF$_{10}$, (γ-lactone), and developed with iso-octane/ethyl acetate/acetic acid/water (25/55/10/50, v/v). Radioactive products were detected with a Packard radiochromatogram scanner, Model 7230. From one plate, zones corresponding to 6-keto PGF$_{10}$, zone I Rf value 0.23, and 6,15-diketo PGF$_{10}$, 6,15-diketo-13,14-dihydro PGF$_{10}$, pentanor PGF$_{10}$, and PGE$_{2}$, zone III Rf values 0.41, 0.47, 0.50, and 0.54 respectively, were cut out, suspended in 10 ml of 0.4% Omnifluor and 20% Triton X-100 in toluene, and counted in a Searle Mark III liquid scintillation counter. The observed cpm were converted to dpm using a quench correction curve and external standards.

From the remaining plates, zones I and III and the zone corresponding to 6-keto PGE$_{1}$ and PGF$_{2\alpha}$, zone II, both of which have an Rf value of 0.36, were scraped from the TLC plate. Following extraction with methanol, zones I and III were prepared for radiometric gas-chromatography and gas-chromatography/mass spectroscopy as described previously.10

Bioassay

The rat stomach strip and bovine coronary artery were arranged in series and superfused with oxygenated (95% O$_{2}$, 5% CO$_{2}$) Krebs' solution at 37°C at a rate of 3 ml/min. Constrictions or relaxations of the tissues were detected using Harvard transducers (type 386) and displayed on a Rikadenki K4-41 pen recorder.

Platelet Studies

Portions of zone II and prostaglandin standards, 6-keto PGE$_{1}$, and PGI$_{2}$, dissolved in isotonic saline, were tested for their musculotropic activity on the assay tissues. Nine parts of whole blood drawn from volunteers who had not taken aspirin or other drugs for the preceding 10 days, were mixed with 1 part of 3.8% w/v sodium citrate. Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared by differential centrifugation, and the final platelet count of PRP was adjusted to 2 X 10$^{10}$/ml with PPP. Then ADP (0.6 μM), L-epinephrine (5.4 μM), and collagen (49 μg) were used to aggregate platelets in 0.5 ml aliquots of PRP stirred at 1200 rpm at 37°C in a dual-channel Payton aggregation module. Changes in light transmission were transcribed on a linear recorder (Payton Associates Buffalo, New York).

Inhibition of ADP-induced platelet aggregation by zone II was compared to that of authentic 6-keto PGE$_{1}$ and PGI$_{2}$. Zone II and prostaglandin standards were diluted in a mixture of ace tone and Tris buffer (1:9), 50 mM, pH 9.5 at 0°C, to the required concentration. Acetone and Tris buffer in this volume and concentration did not affect platelet aggregation.

Materials

Radiolabeled PGI$_{2}$ was synthesized chemically as [9-3H]-PGI$_{2}$ from [9-3H]-prostaglandin F$_{2\alpha}$ (10 Ci/m mole, New England Nuclear, Boston, Massachusetts), and tested for purity by thin-layer chromatography and gas-chromatography/mass spectroscopy according to the method of Johnson et al. The methyl ester of [9-3H]-PGI$_{2}$ was converted to the PGI$_{2}$ sodium salt the day before use by mild alkali hydrolysis.

The 6-keto PGE$_{2}$ was stored in dry acetone at -25°C. Just prior to use, PGI$_{2}$ sodium salt was diluted in Tris buffer to the required concentration. L-epinephrine and ADP were obtained from Sigma (St. Louis, Missouri), and calf-skin collagen was purchased from Calbiochemical Corporation (LaJolla, California).

Results

After extraction and separation, 96% of the infused radioactivity was recovered from the perfusate of the isolated liver of the rabbit. Thin layer chromatography separated the metabolites into zones as described above. Approximately half of the tritiated material migrated in zone I, and a smaller portion was found in zone III.

Derivatization and radiometric gas-chromatography resulted in the separation of the major portion of zone I into a compound that co-chromatographed with 6-keto PGF$_{10}$, and the remainder had the same retention time as 19-hydroxy-6-keto PGF$_{10}$. Similar analysis of zone III revealed radio-labeled metabolites that had the same chromatographic mobilities as dinor-6-keto PGF$_{10}$, pentanor PGF$_{10}$, and pentanor PGE$_{2}$. Mass spectral analyses confirmed the structure of these four metabolites.

After distillation of the aqueous phase, the remaining radioactivity was found to be tritiated water, which accounted for somewhat less than 10% of the total radioactivity of the infused [9-3H]-PGI$_{2}$. Addition of [9-3H]-PGI$_{2}$ to 50 mM Tris buffer, pH 8.9, 37°C, did not result in the formation of radioactive water. The recovery of tritiated water indicates the loss of tritium at the nine position; such would be the case if 6-keto PGE$_{2}$, were a product. Any 6-keto PGE$_{2}$, formed, then, would necessarily be unlabeled and not detectable by radiometric techniques. In the solvent system used, 6-keto PGE$_{2}$, migrated in zone II. When this zone was tested it was found to have biological activity identical to that of authentic 6-keto PGE$_{2}$; viz, it caused constriction of the rat stomach strip and bovine coronary artery (fig. 1 A and B) and was effective in inhibiting ADP-induced platelet aggregation (fig. 1 D and E). The platelet antiaggregatory activity was abolished by prior alkali treatment, as would have been expected if this were 6-keto PGE$_{2}$; alkali pH converts 6-keto PGE$_{2}$, to the biologically inactive prostaglandin, 6-keto PGB$_{2}$. Authentic 6-keto PGE$_{2}$, was found to be equally effective as PGI$_{2}$, over a similar range of doses in inhibiting ADP-induced platelet aggregation (fig. 1 E and F) and likewise
caused contraction of the rat stomach strip (fig. 1 B and C). However, 6-keto PGE₁ differed from PGI₂ in that it caused contraction of the bovine coronary artery (fig. 1 B and C).

In platelet studies using different aggregating agents, 6-keto PGE₁ was found to have equivalent biological effects as PGI₂, both qualitatively and quantitatively. The first phase of epinephrine-induced platelet aggregation was prolonged, and the extent of aggregation was reduced in a dose-dependent manner. With collagen as the aggregatory agent, authentic 6-keto PGE₁ increased the lag time and caused a dose-dependent prolongation of the aggregating period. Furthermore, 6-keto PGE₁, like PGI₂, disaggregated ADP-treated platelets that had already clumped.

Discussion

Hepatic metabolism of PGI₂ in vitro is extensive; β-oxidation accounted for most of the enzymatic conversion of PGI₂. Thus, the loss of a two carbon fragment resulted in the formation of dinor 6-keto PGF₁α, a small proportion of which underwent further oxidation by oxidative decarboxylation to form a pentanor metabolite. Although we did not obtain enough material to identify unequivocally 6-keto PGE₁ as a product of hepatic metabolism of PGI₂ by gas-chromatography-mass spectroscopy, the recovery of material from the liver perfusate with the same chromatographic and biological properties as authentic 6-keto PGE₁ suggests its formation. Further, the recovery of tritiated water provides indirect evidence for the production of 6-keto PGE₁, as, in the course of oxidation to a ketone, the labeled hydrogen ion at the nine position would be lost.

The demonstration in the liver of 9-hydroxy prostaglandin dehydrogenase, enzymic activity that theoretically could convert the hydroxyl group of 6-keto PGF₁α to a ketone, thereby forming 6-keto PGE₁, as shown in figure 2, supports the contention that such a metabolic pathway exists. This enzymic activity has also been described in the kidney. Such an enzymic step is, nevertheless, difficult to reconcile with the lack of biological potency of 6-keto PGF₁α which,
if operational, should transform 6-keto PGF$_{10}$, the inactive product, to 6-keto PGE$_{1}$, unless the amount generated is so small as to be below the threshold required for biological effects. However, the spontaneous hydrolysis product, 6-keto PGF$_{10}$, exists in several isomeric forms. The 6-keto PGF$_{10}$ formed in vivo may not be in the same isomeric form as the synthetically prepared compound. Further, exogenous 6-keto PGF$_{10}$ may not gain access to the same sites as endogenous 6-keto PGF$_{10}$. Alternatively, 6-keto PGE$_{1}$ may be formed from PGI$_{1}$ through an unknown intermediary pathway. Previous studies indicate that metabolism of PGI$_{1}$ by the kidney and mesenteric blood vessels results in different products. Thus, in the kidney the major product is 4, 13-diketo-7, 9, 11-dihydroxy-2,3-dinor prostanoic acid (dinor-6,15-diketo-9,11-dihydroxy prostanoic acid) formed by the successive action of the enzymes 15-hydroxy prostaglandin dehydrogenase, prostaglandin $\Delta^{13}$ reductase, and $\beta$-oxidase. In blood vessels, the only product identified thus far is 6,15-diketo PGF$_{10}$, 15-hydroxy prostaglandin dehydrogenase being the enzyme responsible for this transformation. These various metabolic pathways are summarized in figure 3; they raise the possibility that estimates of PGI$_{1}$ generation may be in error if recovery of 6-keto PGF$_{10}$ is used as the sole index of its synthesis.

Our studies and those of others have demonstrated that 6-keto PGE$_{1}$ is qualitatively and quantitatively similar to PGI$_{1}$ in its effects on platelets, blood
pressure, and renal and pulmonary vascular resistances. These prostaglandins differ in one respect only, in their effects on the bovine coronary artery, which was relaxed by PGI\textsubscript{2} and contracted by 6-keto PGE\textsubscript{1}. As previously reported, 6-keto PGE\textsubscript{1} was found to have the same order of potency as PGI\textsubscript{2} in inhibiting ADP-induced platelet aggregation. The threshold dose and range of doses of 6-keto PGF\textsubscript{1\alpha}, reported to be a potent local vasodilator of the pulmonary vasculature of conscious lambs. The threshold dose for this effect is the same as that for PGI\textsubscript{1}.

It has been shown that PGI\textsubscript{1} is unstable, having a half life of approximately 3 minutes when incubated in aqueous solution at physiological pH and temperature or in blood. Conversely, when 6-keto PGE\textsubscript{1} was incubated in aqueous solution at pH 7.6, 37\degree C, a reduction in its antiaggregatory activity was not seen until at least 15 minutes had elapsed (personal observation). There are several reports of prolonged biological activity of PGI\textsubscript{1}, which are difficult to explain in view of its inherent instability. In human subjects, inhalation of PGI\textsubscript{1} resulted in prolonged resistance of platelets to the proaggregatory action of ADP. In studies using the hamster cheek pouch, ADP-induced thrombi formation had not returned to normal until 30 minutes after the infusion of PGI\textsubscript{1} had been stopped. After the addition of PGI\textsubscript{1} to renal slices, release of renin was stimulated for more than 30 minutes. These observations are consistent with either a sustained response to PGI\textsubscript{1} that persists longer than blood levels of PGI\textsubscript{1} or with transformation of PGI\textsubscript{1} into a more stable substance having prolonged biological activity. We suggest that 6-keto PGE\textsubscript{1} may be generated from either prostacyclin or its stable hydrolysis product, 6-keto PGF\textsubscript{1\alpha}, in the liver and perhaps in other tissues.

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