Prostacyclin, Thromboxane A₂, and Prostaglandin E₂ Formation in Atherosclerotic Human Carotid Artery

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Prostaglandin (PG) formation in 16 atherosclerotic human carotid endarterectomy specimens was compared systematically with that of normal carotid artery from seven white pigs and six rhesus monkeys. Prostacyclin (PGI₂), the major product of arachidonic acid metabolism in normal vascular endothelium, is a potent vasodilator and inhibitor of platelet aggregation and adherence to the vessel wall. It has been hypothesized that PGI₂ counterbalances the proaggregatory and vasoconstrictive effects of predominantly platelet-synthesized thromboxane A₂ (TXA₂). Recent reports suggest that atherosclerotic arteries differ from normal arterial tissues by producing substantially less PGI₂, more PGE₂, and detectable levels of TXA₂. Such alterations in PG synthesis could create a localized imbalance between arterial PGI₂ and platelet TXA₂ with adverse vascular thromboregulatory consequences. (Arteriosclerosis 8:73-78, January/February 1988)

Atherosclerotic Carotid Artery Specimens

Occlusive atherosomatic internal carotid artery plaques were removed from patients (n=16) by standard carotid endarterectomy with appropriate informed consent. Human specimens were obtained in accordance with guidelines established by the Code of Federal Regulations (46.101,b,5) and with approval of the Tulane Medical Center Committee on Use of Human Subjects. Carotid plaques were dissected into the following fractions for systematic analysis of eicosanoid formation (Figure 1): 1) atherosclerotic, but nonatheromatous, intima adjacent proximal (n=10) and distal (n=7) to the occlusive internal carotid plaque; 2) atherosclerotic stenotic intima overlying plaque in the point of arterial narrowing (n=9); specimens that might account for these previously observed disturbances of eicosanoid metabolism and investigate the potential of glutathione (GSH), an endogenous antioxidant, to modulate PG synthesis.

Methods

Normal Carotid Artery Specimens

A single specimen of nonatherosclerotic human carotid artery (n=1) was harvested with appropriate authorization from a brain-dead cadaver kidney donor. Fresh carotid arteries from young adult, white (cross-bred, market grade) pigs (n=7) and rhesus monkeys (n=6) were used as control specimens. The animal experiments conformed to principles of animal care established by the Tulane Medical Center Advisory Committee for Animal Resources, "Principles of Laboratory Animal Care," and the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1978).

Atherosclerotic Carotid Artery Specimens

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ed by addition of 100 μl of 0.1 M potassium phosphate buffer (pH 7.4) and either assayed immediately and those stored for assay at a later date. At the time of analysis, specimens were divided into fractions, blotted dry, weighed while maintaining a temperature of 0°C to 4°C, minced, and then homogenized in potassium phosphate buffer (pH 7.4) and either assayed immediately and those stored for assay at a later date. At the time of analysis, specimens were divided into fractions, blotted dry, weighed while maintaining a temperature of 0°C to 4°C, minced, and then homogenized in a wide range of protein levels and demonstrated increasing PG formation for each specimen is expressed as mean ± standard error of the mean (SEM) in picomoles of stable PG breakdown product formed per 2-minute incubation of PGH2 at each homogenate protein concentration with 2 mM GSH) was determined over a 2-minute incubation of PGH2 at each homogenate protein concentration with 2 mM GSH. Data were analyzed using both parametric and nonparametric methods with comparable results; \( p<0.05 \) was considered statistically significant. One-way analysis of variance with Newman-Keuls post hoc test and the Kruskal-Wallis test were used for group comparisons. Student’s paired \( t \) test and Wilcoxon signed rank test were used to determine the effects of GSH.

**Results**

**Prostacyclin Synthase Activity**

PGI2 synthesis was determined by identification of 6-keto-PGF\(_1\alpha\), the only product of spontaneous PG\(_I\) hydrolysis under these incubation conditions. Basal PGI2 synthase activity (picomoles 6-keto-PGF\(_1\alpha\) formed during 2-minute incubation of PGH2 at each homogenous protein concentration with 2 mM GSH) was determined over a wide range of protein levels and demonstrated increasing PGI2 formation in proportion to the homogenous protein concentration (Figure 2). These data indicate the presence of an active PGI2 synthase in carotid artery specimens. An increase in PGI2 synthase activity was found with the addition of 2 mM GSH at each homogenous protein level (\( p<0.01 \), data not shown).

No difference in PGI2 synthase activity was identified at any protein concentration between normal carotid artery from white pigs or rhesus monkeys and nonatheromatous intima adjacent (proximal or distal) to carotid plaque from atherosclerotic patients (Figure 2) (NS). Basal PGI2 formation (picomoles 6-keto-PGF\(_1\alpha\)/2 min/100 μg homogenous protein with 2 mM GSH) of arteriosclerotic, nonatheromatous, intima adjacent proximal (276 ± 32) and distal (274 ± 32) subintimal plaque (n = 12); and 4) intimal ulceration (n = 4). Not every carotid endarterectomy specimen contained sufficient material from each of these regions for complete determination of PG formation, as noted by the variation in the number of specimens for each fraction. PGI2 synthesis occurs predominantly in the endothelium lining the arterial intima; accordingly, all fractions with a luminal surface were grossly dissected to a thickness of approximately 0.5 mm for comparative purposes.

**Assay of Prostaglandin Formation**

A general discussion of eicosanoid metabolism, PG synthetic pathways, and the pivotal role of PGH2 (the product of arachidonic acid metabolism by cyclooxygenase, and substrate for the terminal enzymes PGI2 synthase in vascular tissues and TX\(_A2\) synthase in platelets) is available from other sources. All of the patients in this study were from other sources.6,7 All of the patients in this study were high risk and were included in the study. A general discussion of eicosanoid metabolism, PG synthetic pathways, and the pivotal role of PGH2 (the product of arachidonic acid metabolism by cyclooxygenase, and substrate for the terminal enzymes PGI2 synthase in vascular tissues and TX\(_A2\) synthase in platelets) is available from other sources.

![Figure 1. Regions of a typical atherosclerotic human carotid endarterectomy specimen; fractions include nonatheromatous intima proximal and distal to internal carotid artery plaque, subintimal plaque, intima in the region of arterial stenosis overlying plaque, and an area of intimal ulceration.](image-url)
(271 ± 14) to the internal carotid plaque was comparable to that of normal carotid from young adult white pigs (272 ± 25) (NS) and rhesus monkeys (219 ± 41) (NS). Data from the single specimen of normal human carotid artery (not entered into the statistical analysis) indicate values of PGI₂ formation also within the range of these specimens.

Figure 3 shows the 6-keto-PGF₁α formation by fractions of human carotid endarterectomy specimens at various homogenate protein concentrations. PGI₂ formation by nonatheromatous intima proximal and distal to carotid plaque was similar at all protein concentrations (NS, data not shown). A decrement in PGI₂ formation was demonstrated between nonatheromatous intima adjacent proximal or distal to the carotid plaque and stenotic intima overlying the plaque, subintimal plaque, and areas of intimal ulceration. It should be noted that PGI₂ formation in subintimal plaque was comparable to stenotic intima overlying plaque at the point of arterial narrowing; the least activity was found in intimal ulcerations. Other than areas of ulceration, all specimens increased PGI₂ formation with the addition of 2 mM GSH (p<0.05) (Table 1).

**Thromboxane A₂ Synthase Activity**

TXA₂ formation determined by identification of TXB₂, the stable product of TXA₂ hydrolysis, was not detected at any protein concentration in homogenates of white pigs, rhesus monkeys, or human carotid artery specimens, and if present was, thus, less than 10 picomoles.

**Glutathione-Dependent Prostaglandin E₂ Isomerase Activity**

Data in Tables 1 and 2 demonstrate an inverse relationship between PGI₂ and PGE₂ formation for all carotid artery specimens in both degree of plaque pathology and homogenate protein concentration. In all cases in which PGI₂ formation was low, PGE₂ formation was high; a reciprocal relationship was observed when PGI₂ formation was high. PGE₂ formation decreased with increasing homogenate protein concentration, although no difference in PGE₂ formation by carotid artery from white pigs, rhesus monkeys, or human specimens was found at any given protein concentration (NS). Additionally, no increase in PGE₂ formation was detected after addition of 2 mM GSH (NS).

**Discussion**

This study demonstrated the presence of an active PGI₂ synthase in carotid artery from white pigs, rhesus monkeys, and in human normal and atherosclerotic tissue. Nonatheromatous arterial intima adjacent to atherosclerotic carotid plaque formed PGI₂ in quantities indistinguishable from that of normal carotid artery specimens from young adult white pigs and rhesus monkeys. In contrast, atheromatous fractions of human carotid plaque formed significantly less PGI₂ than adjacent arteriosclerotic, nonatheromatous, arterial intima and was least in areas of plaque ulceration. Although other authors³⁴ have reported decreased PGI₂ formation relative to the severity of atherosclerosis, this study was the first to systematically evaluate
the focal nature of decreased PGI$_2$ formation in complex atheromatous carotid specimens.

A number of cells in the arterial wall (endothelium and smooth muscle) are capable of PGI$_2$ synthesis. Considerable variation in the distribution of cellular elements within advanced human carotid plaques was recently reported. Our data demonstrate that decreased PGI$_2$ formation in patients with advanced atherosclerosis is focal (i.e., localized to atheromatous plaque) and not a consequence of generalized arteriosclerosis. The data do not indicate whether decrements in PGI$_2$ formation observed in atherosclerotic fractions resulted from a less active PGI$_2$ synthase, cellular heterogeneity, or dilution of PGI$_2$ synthase by atheromatous material.

Demonstration of PGI$_2$ synthesizing capability in human and experimental arteriosclerosis is not without controversy. Diminished PGI$_2$ formation in atherosclerotic specimens has been reported previously but other studies have found insignificant differences or increases. The significance of in vitro studies of PGI$_2$ formation by atherosclerotic arterial specimens has recently been questioned. Quantitative correlation between the in vitro capacity of vascular tissues to synthesize eicosanoids and actual endogenous biosynthetic rates may be tenuous, and discrepancies between the two have been suggested. Direct in vivo assay of PGI$_2$ formation by atherosclerotic arteries is not currently possible and, thus, other authors have investigated endogenous PGI$_2$ synthesis by indirect methods. Basal production of PGI$_2$ under physiologic conditions is reported to be low. Patients with severe atherosclerosis and evidence of platelet activation were observed to excrete higher urinary levels of 2,3-dinor-6-keto-PGF$_{1\alpha}$ (one of a number of in vivo PGI$_2$ metabolites) than normal subjects; these authors concluded that reduced PGI$_2$ formation by atherosclerotic specimens observed in vitro was, therefore, physiologically irrelevant. It was proposed that endogenous production of PGI$_2$ was lower than normal vascular tissues are capable of producing because of the lack of a stimulus and, conversely, higher in atherosclerotic patients. Possible mechanisms for stimulation of PGI$_2$ formation (increased platelet/vascular interactions) are speculative.

Increased urinary excretion of a PGI$_2$ metabolite from unknown tissue origin in atherosclerotic patients and studies indicating a localized reduction in PGI$_2$ synthesis by atheromatous plaques are not necessarily contradictory. Whether reported in vivo increases in PGI$_2$ formation in patients with advanced atherosclerosis occur in atheromatous or nonatheromatous arterial regions is currently unknown and cannot be determined conclusively by indirect assay methods. If, as suggested by urinary PGI$_2$ metabolite excretion studies, increased PGI$_2$ is produced in atherosclerotic patients, the site of PGI$_2$ formation may not be from plaques themselves, but from neighboring nonatheromatous arterial intima. Definitive conclusions regarding endogenous vascular PGI$_2$ biosynthesis in atherosclerotic patients, based on a quantitative correlation with urinary eicosanoid metabolite levels, may be premature.

Deficiencies in PGI$_2$ synthase activity may set limits on endogenous PGI$_2$ biosynthesis by atheromatous plaques.
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regardless of the level of stimulation. Although the theory is speculative and requires further experimentation, the nonatheromatous portions of the arterial system may be subjected to increased traumatic or chemically mediated platelet/vascular interactions by proximity to neighboring plaques and, thus, stimulated to produce PG12.13,14,17 Additionally, hemodynamic factors such as increased flow velocity or wall shear stress in nonatheromatous regions adjacent to atherosclerotic arterial stenoses could stimulate PG12 formation.18,19 Whether PG12 synthesis by nonatheromatous intima can regulate platelet adherence to nearby plaques is currently unknown. Flow separation and other complex fluid disturbances at the carotid bifurcation and in the proximity of occlusive plaques may interfere with the local dispersion of PG12 in the blood stream, precluding inhibition of platelet aggregation and adherence.20,21 It has been shown in vitro that small concentrations of PG12 prevent thrombus formation, but larger concentrations are needed to reduce platelet adhesion.9 Imbalances between PG12 and TXA2 could have localized adverse thromboregulatory consequences in the immediate environment of atherosclerotic plaques, not necessarily reflected in circulating or urinary PG12 metabolites.

It has been proposed that lipid peroxides or other reactive oxygen species found in atherosclerotic vessels inhibit PG12 formation. PG12 synthase is uniquely sensitive to oxidant (O2-) released after peroxidation of 15-hydroperoxy-icosatetraenoic acid (15-HPETE), a lipoxygenase product of arachidonic acid found in atherosclerotic plaques.22 A recent study from our laboratory found that PG12 formation in coronary artery microsomes could be modulated in a dose-dependent manner by GSH.8 We suggested that PG12 synthase activity determinations with the addition of GSH are more reliable indicators of basal PG12 synthase activity, because GSH is present intracellularly in millimolar concentrations.6 Both normal and atherosclerotic carotid artery formed more PG12 in the presence of GSH; however, GSH was unable to restore PG12 formation in carotid plaque to a level comparable to that of the surrounding nonatheromatous intima. These data suggest that a reduction in, or loss of, the effectiveness of intracellular GSH to maintain basal PG12 synthase activity as a consequence of oxidative or other mechanisms could contribute to diminished PG12 formation.

TXA2 synthesis by arterial tissues is controversial, but TXA2 formation by whole atherosclerotic human carotid plaque specimens has been recently reported.3 We found no detectable TXA2 formation in any normal or atherosclerotic carotid artery homogenates. Because of the restricted quantity of material in each carotid plaque specimen, it is possible that homogenate protein concentrations used in this study were too low to support detectable levels (10 picomoles) of TXA2 formation by radiometric TLC. Nevertheless, current data suggest that neither normal nor atherosclerotic carotid artery have substantial capability to form TXA2. Arterial TXA2 formation is, thus, unlikely to be a major contributing factor in platelet adherence to carotid plaque.

The reciprocal relationship between PG12 and PGE2 formation has been observed previously in atherosclerotic human aorta.4 It was proposed that atherosclerotic aorta shifts eicosanoid metabolism from PG12 synthesis to produce PGE2 predominantly.4 PGE2 formation can occur by spontaneous hydrolysis of PGH2 or enzymatically by GSH-dependent PGE2 isomerization of PGH2.8 Our data demonstrate substantially less PGE2 formation when PGH2 is converted rapidly to PG12 by PG12 synthase, and vice versa; additionally, no GSH-dependent PGF2 formation was observed. We conclude that in carotid artery homogenates most, if not all, PGE2 was formed nonenzymatically secondary to spontaneous hydrolysis of PGH2. The significance of this apparent lack of GSH-dependent PGE2 isomerase in carotid artery is currently unknown, but could preclude substantial endogenous PGE2 formation. PGE2 reportedly promotes the second phase of platelet aggregation.25 While it is doubtful that unregulated PGE2 formation by spontaneous hydrolysis of PGH2 contributes to carotid arterial thromboregulatory mechanisms, PGE2 formed nonenzymatically could oppose platelet inhibitory effects of PG12. Previous work in our laboratory, which unmasked the presence of GSH-dependent PGE2 isomerase activity in bovine coronary artery microsomes, demonstrated the need for appropriately controlled experiments before the presence of GSH-dependent PGE2 isomerase in vascular tissues could be definitely excluded.8,14,15 A study reports significant focal alterations in eicosanoid metabolism, particularly diminished PG12 formation, in lipid to atheromatous fractions of human carotid endarterectomy specimens. Adjacent arteriosclerotic, but nonatheromatous, arterial intima formed PG12 in quantities indistinguishable from that of normal human and animal control specimens. GSH, an endogenous antioxidant, was shown to modulate PG12 formation in all carotid artery specimens but did not restore PG12 formation in atheromatous fractions to basal level. Although the overall consequences on the vascular system are unknown, focal decrements in PG12 synthesis by atherosclerotic carotid plaques could have a profound impact on arterial thromboregulatory functions in patients with extracranial carotid atherosclerosis.

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