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Increased Prostacyclin and Thromboxane A_2 Formation in Human Varicose Veins[1]

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Increased urinary metabolites of the antiaggregatory vasodilator prostacyclin (PGI_2) and the proaggregatory vasoconstrictor thromboxane A_2 (TXA_2) have been reported in deep vein thrombosis; however, the tissue(s) of origin is uncertain. Because little is known about the formation of PGI_2 or TXA_2 from its common precursor, prostaglandin (PG) endoperoxide H_2 (PGH_2), by varicose veins, we determined the formation of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2), TXB_2 (the stable metabolite of TXA_2), and PGE_2. Segments of normal saphenous vein and varicose vein (nine and six patients, respectively) were incubated with 10 μM [^{14}C]PGH_2 for 2 min at 37°C; products were separated by thin-layer chromatography. Surface area and mass of normal and varicose vascular segments were 19.5 ± 0.8 versus 18.8 ± 0.6 mm^2 and 11.6 ± 1.4 versus 10.7 ± 0.7 mg, respectively. Formation of 6-keto-$PGF_{1\alpha}$ and TXB_2 by the segments of varicose vein was significantly increased over that of normal vein: 157 ± 14 versus 243 ± 17 pmole of 6-keto-$PGF_{1\alpha}$ ($P < 0.005$) and 22 ± 3 versus 35 ± 5 pmole of TXB_2 ($P < 0.01$). The formation of PGE_2 by segments of varicose vein was not significantly different from that of normal vein (201 ± 9 vs 219 ± 11, respectively). Deoxyribonucleic acid (DNA) content of normal and varicose vein was 1.69 ± 0.12 and 1.51 ± 0.13 mg per gram of tissue, respectively. The data suggest that the increased PGI_2 formation may reflect increased activity or content of PGI_2 synthase. The increase in TXA_2 formation may reflect increased productivity or an increased presence of residual platelets or microemboli.

INTRODUCTION

Arachidonic acid, a 20-carbon polyunsaturated fatty acid, is a constituent of the phospholipid component of mammalian cell membranes. Release of arachidonic acid from the phospholipid pool is catalyzed by phospholipase and can result from a number of interventions including receptor-mediated events, such as stimulation by bradykinin or histamine, and non-receptor-mediated events, such as tissue injury. Prostaglandin (PG) H synthase is an enzyme found in many cell types that catalyzes the formation of PGG_2 from arachidonic acid (cyclooxygenase step), as well as the subsequent conversion of PGG_2 to PGH_2 (peroxidase step). PGH_2 is a pivotal substance that can be converted to prostacyclin (PGI_2), PGE_2, PGD_2, $PGF_{2\alpha}$, and thromboxane (TXA_2). The metabolism of PGH_2 is tissue specific; however, all the metabolites of PGH_2 are biologically active. Vascular tissue, both endothelium and smooth muscle, form PGI_2 and have been reported to form TXA_2 in small amounts, whereas the platelet principally converts PGH_2 only to TXA_2. Vascular formation of PGI_2 and platelet-generated TXA_2 are thought to be important modulators of thrombogenesis, because PGI_2 is an inhibitor of platelet adhesion to the blood vessel as well as platelet aggregation, whereas TXA_2 induces platelet aggregation [20]. PGE_2 has been reported to produce vasodilation in the systemic vascular bed [15] and to modulate platelet aggregation [24]. We have previously demonstrated the presence of an active GSH-dependent PGH_2-to-PGE_2 isomerase in human saphenous vein [16].

The human saphenous vein is used widely as an autologous graft in vascular surgery. It is used commonly in revascularization of the coronary arteries and lower limbs and for repair of traumatic arterial and venous injuries elsewhere in the body. Considerable research has focused on the physiologic mechanisms of graft patency and on the role of the eicosanoid PGI_2 [3, 5–7, 16, 22]. Because PGI_2 is produced by both intimal and subintimal regions of the vascular wall [17, 18, 26] and functions as a local vasodilator and inhibitor of platelet aggregation, it has been postulated that an increased ratio of PGI_2 to the proaggregatory compound TXA_2 prolongs the patency of venous grafts [6].

Recent data have shown that PGI_2 formation is augmented by increased intraluminal pressure and pulsatility

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TABLE 1

Donor Source of Saphenous Vein Specimens

Normal saphenous veins	n^a	Varicose saphenous veins	
Femoral-popliteal bypass graft	4	Patients undergoing varicose vein stripping procedures	6
Coronary artery bypass graft	3		
Arterial reconstruction for trauma	1		
Vein patch angioplasty	1		

[a] n = number of donors.

of flow [5–7], as well as stretching of the endothelial cell layer [26]. Trauma to the vessel wall also causes PGI_2 release [14]. Moreover, increased urinary metabolites of PGI_2 and TXA_2, as well as increased synthesis of these two substances in hand veins, have been reported in patients with deep venous thrombosis [23, 27]; the tissue responsible for increased production of these substances is unknown. Thus, the literature suggests the hypothesis that there may be alterations in the metabolism of PGH_2 by vascular tissue exposed to increased extraluminal pressure. The purpose of this study was to investigate the differences in eicosanoid production between normal saphenous veins and varicose saphenous veins, which are subjected to both increased pressure and stasis of blood flow.

METHODS

Normal saphenous veins were obtained from three sources: patients undergoing coronary artery bypass grafting; patients undergoing femoral–popliteal or femoral–tibial artery bypass using reversed or *in situ* saphenous vein; and patients in whom the saphenous vein was used as a vein patch or interposition graft in repair of vascular trauma (Table 1). In cardiac and trauma patients, the harvested vein was kept in iced 0.1 *M* potassium phosphate buffer, pH 7.4 until completion of anastomosis (15 to 60 min); leftover vein was then placed in 0.1 *M* potassium phosphate buffer, pH 7.4, and frozen at −40°C for later analysis. In lower-extremity bypass patients, sections of vein were carefully dissected at the start of the procedure and then frozen in buffer at −40°C for later analysis.

Varicose saphenous veins were obtained from patients undergoing vein stripping for removal of painful or unsightly varicosities of the legs. Segments of varicose saphenous vein were dissected from the lower leg at the level of the ankle and frozen in buffer for later analysis; the remainder of the vein removal was performed using conventional stripping wires.

To cut precise rings, the blood vessels were laid on a movable stage beneath a razor blade guillotine and cut to provide an 18- to 20-mm^2 intimal surface area. The segments were placed in phosphate-buffer solution and warmed to 37°C in a water bath for 2 min. The warmed segments and buffer were then added to an incubation tube containing [^{14}C]PGH_2, which had been previously blown to dryness under a stream of N_2 and incubated for 2 min at 37°C while being stirred with a magnetic stir bar. The radiolabeled PGH_2 was prepared from radiolabeled arachidonic acid in our laboratory using a protocol published previously [19]. The reaction was stopped and remaining substrate and radiolabeled products were then extracted by addition of ethyl acetate:methanol:0.2 *M* citric acid, pH 2 (15:2:1). The reaction tubes were vortexed and centrifuged at 12,000*g* for 30 sec. The eicosanoids in the organic upper layer were separated by thin-layer chromatography (TLC). Products were identified by comparison with the migration of authentic standards, which were located by iodination of the chromatography plates. Radioactivity on the TLC plates was monitored by radiochromatogram scanners mated to Commodore 64 computers for data storage, analysis, and calculation. PGI_2 was identified through the formation of its spontaneous breakdown product, 6-keto-$PGF_{1\alpha}$; TXA_2 was identified as its breakdown product TXB_2. Duplicate assays were performed on each specimen. These procedures are similar to those published previously [18].

In another experiment, segments of vein were prepared from the area immediately adjacent to the segments that were incubated. These segments were analyzed for deoxyribonucleic acid (DNA) content using the method described by Fiszer-Szafarz *et al.* [10].

PG formation is expressed as the mean (± standard error of the mean) picomoles of stable PG breakdown product formed per 2 min incubation of PGH_2. For statistical analysis, P values were calculated using Student's t test, with $P < 0.05$ considered statistically significant.

RESULTS

Segments of both normal and varicose saphenous veins were prepared as described in the Methods to ensure minimal differences between them with respect to mass and

TABLE 2

Surface Area and Mass of Human Saphenous Vein Segments

Saphenous vein	n^a	Surface area (mm^2)	Mass (mg)
Normal	9	19.5 ± 0.8	11.6 ± 1.4
Varicose	6	18.8 ± 0.6	10.7 ± 0.7

[a] n = number of donors.

surface area. The data presented in Table 2 indicate that the segments prepared from normal and varicose veins did not differ significantly from each other with respect to either of these values.

When segments of either normal or varicose vein were incubated with PGH_2, the immediate precursor of both PGI_2 and TXA_2, both normal and varicose venous segments formed 6-keto-$PGF_{1\alpha}$ and TXB_2 (the stable hydrolysis products of chemically unstable PGI_2 and TXA_2, respectively), as well as PGE_2 (Table 3). The quantity of TXB_2 produced by normal saphenous vein segments was close to the limit of detection of the method used (i.e., 10 pmole). Statistically significant differences between the normal and varicose venous segments were observed, however, with the varicose segments producing 55% more 6-keto-$PGF_{1\alpha}$ ($P < 0.002$) and 59% more TXB_2 ($P < 0.03$) than the normal segments. When these data were expressed as the ratio 6-keto-$PGF_{1\alpha}$/TXB_2, little difference was noted between the normal and varicose venous segments. No statistically significant difference was found in PGE_2 formation between normal and varicose venous segments.

The DNA content of the venous segments prepared from the area immediately adjacent to the segments, which were used to study the formation of 6-keto-$PGF_{1\alpha}$ and TXB_2, was determined. The data in Table 4 indicate no statistically significant difference in the DNA content between segments prepared from normal or varicose human saphenous vein.

DISCUSSION

The data indicate that segments of varicose saphenous veins produce significantly more PGI_2 and TXA_2 from PGH_2 *in vitro* than segments of normal saphenous veins, whereas the formation of PGE_2 is similar in varicose and normal vein tissue. The vascular segments were prepared in a manner to minimize physical differences between normal and varicose vascular segments, and the data indicate no significant difference between the segments [illegible] in this study, based on mass or surface area. It is unlikely that differences in age or sex of the patient are involved because it has been reported that neither 6-keto-[illegible] nor TXB_2 formation by segments of hand vein from patients with deep vein thrombosis varied with age or sex [14]. The quantity of 6-keto-$PGF_{1\alpha}$ produced by normal vein in this study (157 ± 14 pmole) is similar to that reported to be produced by normal saphenous vein segments of similar mass (165 ± 9 pmole) in an earlier study (1); the quantity of 6-keto-$PGF_{1\alpha}$ produced by varicose vein in this study (243 ± 17 pmole) is greater than that observed previously for normal human aorta (20 ± 16 pmole) [1].

Possible mechanisms that may underlie the increase in PGH_2 metabolism include increased specific activity of PGI_2 synthase; increased amount of enzyme per cell (*de novo* synthesis of PGI_2 synthase); and hyperplasia resulting in an increased number of cells contributing to the metabolism of PGH_2. We therefore determined the DNA content of segments of normal and varicose saphenous veins. No significant difference was found in the DNA content between the segments used in this study, indicating no significant difference in the number of cells between normal and varicose vascular segments. These data suggest that the observed increase in the production of 6-keto-$PGF_{1\alpha}$ may reflect increased activity per cell of PGI_2 synthase, rather than hyperplasia resulting in an increased number of cells contributing to the formation of 6-keto-$PGF_{1\alpha}$. The data, however, do not preclude the possibility of differences in cell type, with the increased metabolism of PGH_2 reflecting an altered proportion of cells that metabolize PGH_2 between normal and varicose vascular segments.

With respect to TXA_2 formation, the quantity formed (22 ± 3 pmole) by normal saphenous vein is small and close to the limit of detection of the method (10 pmole). In an earlier study, we found that normal saphenous vein did not produce detectable amounts of TXA_2 [1]. Thus, the normal saphenous vein produces only a minimal quantity of detectable TXA_2. The statistically significant increase in TXB_2 formation and significant increase in DNA content is consistent

TABLE 3

Prostacyclin and Thromboxane A_3 Formation from PGH_2 by Segments of Normal and Varicose Human Saphenous Vein

Saphenous vein	*n*	6-Keto-$PGF_{1\alpha}$	TXB_2	6-Keto-$PGF_{1\alpha}$/TXB_2	PGE_2
Normal	9	157 ± 14	22 ± 3	7.1	219 ± 11
Varicose	6	243 ± 17*	35 ± 5**	6.9	201 ± 9

Note. Conditions of incubation: 0.1 *M* potassium phosphate, pH 7.4; 10 μM PGH_2; 37°C; 2 min. Data are expressed as picomoles of product formed and are means ± SEM of duplicate incubations of vascular segments prepared from each donor; *n* = number of donors.

* $P < 0.005$ compared with normal.

** $P < 0.01$ compared with normal.

TABLE 4

Deoxyribonucleic Acid (DNA) Content of Normal and Varicose Human Saphenous Veins

Saphenous vein	*n*	DNA (mg/g tissue)
Normal	8	1.69 ± 0.12
Varicose	5	1.51 ± 0.13

Note. Data are expressed as means ± SEM of determinations made on vascular segments prepared as described under Methods; *n* = number of donors. $P < 0.36$.

the presence of a small quantity of platelets or microaggregates in the segments of varicose vein. This possibility, however, cannot be distinguished by the present data from an increase in either specific activity or quantity of TXA_2 synthase as suggested for PGI_2 synthase, because the platelet does not contain DNA.

Although an increase in the formation of PGI_2 and TXA_2 by varicose saphenous veins was found, the ratio of PGI_2 to TXA_2 (6-keto-$PGF_{1\alpha}$/TXB_2) was similar between normal and varicose vascular segments. These data indicate no imbalance in the ratio associated with varicose veins. This observation is similar to that reported by Saldeen *et al.* [23] in that the increased synthesis of 6-keto-$PGF_{1\alpha}$ and TXB_2 by segments of hand veins from patients with deep venous thrombosis on gentle shaking in a water bath was not associated with an altered 6-keto-$PGF_{1\alpha}$/TXB_2 ratio. Because PGI_2 has a half-life of approximately 4 to 5 min, whereas TXA_2 has a half-life of approximately 30 sec, the functional significance of the increase in PGI_2 formation of an average of 86 pmole accompanied by an increase of an average of 13 pmole in TXA_2 by the same vascular segment is uncertain. Although TXA_2 has been suggested to be more potent on a molar basis than PGI_2, no direct evidence exists [2].

The relationship between varicose veins and increased intraluminal pressure is well established. A contributing anatomic defect is the incompetence of venous valves; whether this is the primary cause of varicose change or rather a result of the postphlebitic syndrome and dilatation is uncertain [8]. Histologically, varicose veins demonstrate variations in wall thickness, with areas of elastic tissue degeneration and areas of smooth muscle hypertrophy [21]. Areas of distension and aneurysmal outpouching are common. These changes combine with valvular insufficiency to allow pooling of blood in the veins, which, in turn, can promote thrombosis, edema, and venous ulceration. PGI_2 acts to inhibit thrombosis and has been postulated to exert *in vivo* a limitation on clot propagation, confining the clot to the site of vascular injury [13]. Normal veins placed into an arterial environment increased PGI_2 formation [7]. These arterialized veins roughly double their basal PGI_2 production, such that it approximates the basal level of native artery. Our data indicate that the PGH_2 metabolism of varicose vein segments is altered under these conditions of increased risk of thrombosis in a manner that results in increased production of both PGI_2 and TXA_2. PGI_2 formation by varicose vein increased to levels greater than that observed previously in normal human aorta [1]. Whether this increase in PGI_2 formation is a result of endothelial stretching and increased pressure or a response to some other signal is unknown.

With respect to endothelial PGI_2 formation, PGI_2 is formed by endothelial as well as vascular smooth muscle cells [17, 18]. A gradient in cyclooxygenase activity in which arachidonic acid metabolism is greatest in the intima (endothelial) compared with subintima (smooth muscle) has been reported; however, there does not appear to be such a gradient in PGI_2 synthase activity [9, 18]. As such, vascular production of PGH_2 from arachidonic acid is more dependent on an intact endothelium than is the conversion of PGH_2 to PGI_2 [18]. Thus, in this study, the role of the endothelium in formation of PGI_2 by normal versus varicose segments is not addressed, because PGH_2, not arachidonic acid, was used as substrate.

PGI_2 has been shown by other investigators to have different effects on the arterial and venous systems [11, 25, 28]. While PGI_2 has been reported to be a potent arterial vasodilator, it has been reported to be without effect in the bovine intrapulmonary venous rings [12] and to produce a contractile response in both normal and varicose human saphenous vein strips [25]. If PGI_2 produces a venoconstrictor response *in vivo* in the varicose vein, this would be potentially beneficial, because a decrease in luminal diameter could augment venous return.

More recently, Biagi *et al.* reported that human varicose saphenous vein produced less PGI_2 and more TXA_2 and PGE_2 than normal saphenous vein [4]. As discussed above, we have previously demonstrated a gradient in PGI_2 formation from arachidonic acid but not PGH_2 by vascular segments [18]. The differences between the data of Biagi *et al.* and the present data suggest that the endothelial layer of varicose saphenous vein may be damaged, resulting in decreased arachidonic acid conversion to PGI_2; however, further work is required to more fully elucidate this point.

In conclusion, vascular segments prepared from varicose human saphenous vein produce increased amounts of PGI_2 and TXA_2. The relationship between venous hypertension associated with varicose veins and increased formation of these eicosanoids requires further investigation. In addition, the physiologic significance of the increased formation of PGI_2 and TXA_2 with respect to contractile response of the vein and platelet aggregation remains to be determined.

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