Alterations of Thrombogenesis, Endothelial Damage and Oxidative Stress with Reperfusion during Femoral Artery Bypass Surgery for Peripheral Vascular Disease

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Key Words

Peripheral vascular disease · Thrombogenesis · Angiogenesis · Oxidative stress · Endothelium

Abstract

Peripheral vascular disease (PVD) is a significant cause of cardiovascular morbidity. We hypothesised that there would be significant alterations of thrombogenesis, platelet activation and endothelial damage, which could be associated with abnormal oxidative stress during femoral artery bypass surgery for PVD, where the femoral artery is cross-clamped (causing acute ischaemia) and reperfused (following revascularisation). To test this hypothesis, we measured sequential changes in von Willebrand factor (vWF, and index of endothelial damage/dysfunction), tissue factor (TF, an index of thrombogenesis) and soluble P-selectin (sP-sel, an index of platelet activation) as well as lipid hydroperoxides (LPO, an index of oxidative stress) in 28 consecutive patients undergoing elective peripheral artery bypass surgery. Mean baseline vWF and sP-sel levels in PVD patients (before clamping) were significantly higher compared with age- and sex-matched controls (unpaired t test, both $p < 0.05$),

but there were no significant differences in TF and LPO levels. There was a correlation between TF and vWF (Spearman's, $r = 0.374$, $p = 0.05$), as well as between sP-sel and vWF at the start of surgery ($r = 0.467$, $p = 0.012$). The patients undergoing peripheral artery bypass surgery had a mean femoral artery clamp time of 28 min (standard deviation 14 min; range 11–65 min). There were no significant overall changes in sP-sel, vWF, TF and LPO with femoral artery cross-clamping and reperfusion (repeated measures ANOVA, $p = \text{NS}$). In conclusion, we found that during ischaemia-reperfusion during peripheral arterial bypass surgery, thrombogenesis (as measured by plasma TF) and oxidative damage (as measured by LPO) within the affected leg does not increase in the immediate perioperative period. Further studies are required to assess the mechanism(s) of ischaemia-reperfusion injury in PVD, and the contributory role(s) of the endothelium and platelets.

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Introduction

Peripheral vascular disease (PVD) is clinically detectable in approximately 29% of people aged 55–74 of whom 4.5% have intermittent claudication, the most common clinical manifestation of PVD [1], and approximately 5% of patients with intermittent claudication will develop critical limb ischaemia requiring intervention in 5 years [5]. Three options are currently available for intervention: angioplasty (with or without intraluminal stenting), peripheral artery bypass and amputation.

Peripheral artery bypass surgery carries with it significant morbidity and mortality, with the most risky procedure being aorto-bifemoral bypass with an operative mortality of 3% [3]. Other common complications associated with peripheral artery surgery include graft thrombosis, myocardial infarction and stroke, all of which have thrombosis as the underlying pathophysiological process.

Recent advances in laboratory techniques have enabled the quantification of components of Virchow's triad, leading to thrombus formation (thrombogenesis). For example, von Willebrand factor (vWF) is an established index of endothelial damage/dysfunction, which is increased in PVD [4–7]. Another marker, tissue factor (TF), is an index of a prothrombotic state, as under normal physiological conditions, TF is expressed only on extravascular sites and perivascularly in the adventitial layer of blood vessels, but raised TF has been found in patients with proven PVD compared to controls [8]. Platelet activation is also a crucial component of thrombogenesis, and may be quantified by measurement of soluble P-selectin (sP-sel) [9].

Whilst abnormal vWF, TF and sP-sel levels have been found in atherosclerosis [10–13], the effects of acute ischaemia (for example, following cross-clamping for vascular surgery) on these indices are not known. Oxygen free radicals are responsible for oxidative damage of tissues exposed to ischaemia, although the changes following balloon angioplasty and surgery for PVD have been conflicting [14–16]. It remains uncertain whether the changes in oxidative stress contribute to abnormal thrombogenesis following vascular surgery.

We hypothesised that there would be significant alterations of thrombogenesis, platelet activation and endothelial damage, which could be associated with abnormal oxidative stress during femoral artery bypass surgery for PVD, where the femoral artery is cross-clamped (causing acute ischaemia) and reperfused (following revascularisation). To test this hypothesis, we measured sequential changes in vWF, TF and sP-sel, as well as lipid hydro-

peroxides (LPO, an index of oxidative stress) in patients undergoing lower limb surgical revascularisation.

Patients and Methods

We recruited 28 consecutive patients who were undergoing elective peripheral artery bypass surgery. Demographic data including risk factors, past medical history and drug history, as well as an electrocardiogram and standard laboratory tests were recorded preoperatively. Patients underwent the usual preoperative work-up and anaesthesia, either epidural with propofol sedation or general anaesthesia. Active fluid management was continued throughout the surgery. As per current clinical guidelines, all patients were established on aspirin (>3 months) prior to surgery. We excluded patients with recent (<6 weeks) myocardial infarction, unstable angina, stroke or congestive heart failure, as well as those with renal or liver impairment and warfarin therapy. The West Birmingham Ethics Committee passed the protocol and informed consent was obtained.

During surgery, blood samples were obtained from the femoral vein of the operated side by direct, separate puncture with a 21-gauge needle and syringe, under direct visualisation. The sample was taken at the following time points: (i) immediately before the inflow (common femoral artery) clamp was applied ('baseline'); (ii) just before it was released, and (iii) 5 min and (iv) 30 min after it was released. Baseline results were compared with age- and sex-matched healthy controls, recruited from hospital staff and preoperative clinics for minor procedures, e.g. varicose veins or cataract surgery. All healthy controls were 'healthy' by virtue of careful clinical history and examination, as well as basic blood screening tests.

Laboratory

Serum and citrated plasma were obtained and frozen at -70°C for batch analysis. Analysis for sP-sel (R & D Systems, Abingdon, UK), vWF (Dako, Copenhagen, Denmark) and TF (Axis-Shield, Dundee, UK) was performed by ELISA. LPO were determined by the ferrous oxidation of the colorimetric dye xylenol orange at a wavelength of 560 nm in conjunction with the specific hydroperoxide discriminant triphenylphosphine as described by Nourooz-Zadeh et al. [17]. Intra-assay and interassay variances of all assays were 5 and 10%, respectively.

Power Calculations

We hypothesized that values of sP-sel, vWF, TF and LPO would be increased by one half of a standard deviation (SD) between the time points of the study in patients undergoing bypass surgery. This would require a minimum of 26 patients to complete the study with a power of 80% and a significance of <0.05 .

Statistical Analyses

Normality tests were performed on all data. Parametric data are expressed as mean (SD) and nonparametric data as median (interquartile range). Comparisons between cases and controls were performed with the unpaired *t* test and Mann-Whitney *U* test, as appropriate, whilst the effects of surgery were assessed using repeated measures ANOVA. Correlations between various indices were performed using Spearman's rank correlation. A probability of <0.05 was considered as statistically significant.

Table 1. Characteristics of patients with PVD and controls

	PVD (n = 28)	Healthy controls (n = 50)	p value
Age, years	72 ± 10	69 ± 10	0.132
Males	24	32	0.08
Past medical history			
IHD or MI	9	0	<0.001
Current smoker	15	15	0.04
Treated hypertensive	18	0	<0.001
Treated hypercholesterolaemia	6	0	<0.001
Diabetic	7	0	<0.001
CVA or TIA	6	0	<0.001
Systolic blood pressure, mm Hg	146 ± 26	138 ± 13	0.021
Diastolic blood pressure, mm Hg	78 ± 12	79 ± 8	0.859
vWF, IU/dl	139 (23)	109 (29)	0.03
sP-sel, ng/ml	48 (21)	40 (10)	0.025
TF, pg/ml	10 (10–25)	15 (10–50)	0.564
LPO, µmol/l	8.1 (5.6–11.5)	7.5 (4.5–12.2)	0.743

Values are means ± SD or medians (interquartile range). Analysis by unpaired t test or χ^2 test, as appropriate. IHD = Ischaemic heart disease; MI = myocardial infarction; CVA or TIA = cerebrovascular accident or transient ischaemic attack.

Results

The demographic features of patients and controls included in the study are shown in table 1. Mean baseline vWF and sP-sel levels in PVD patients (before clamping) were significantly higher compared with age- and sex-matched controls (unpaired t test, both $p < 0.05$; table 2, fig. 1, 2). There were no significant differences in TF and LPO levels (fig. 3, 4).

Table 2 shows the correlations between the baseline values for the three tests performed as well as for the lowest ankle brachial pressure index of the patients undergoing surgery. There was a correlation between TF and vWF ($p = 0.05$), as well as sP-sel and vWF at the start of surgery (Spearman's, $p < 0.05$).

The patients undergoing peripheral artery bypass surgery had a mean femoral artery clamp time of 28 min (SD 14 min; range 11–65 min). There were no significant overall changes in sP-sel, vWF, TF and LPO with femoral artery cross-clamping and reperfusion (repeated measures ANOVA, $p = \text{NS}$; table 3).

Table 2. Spearman's correlations between baseline variables

		Correlation	p value
Lowest ABPI vs.	LPO	−0.041	0.851
	TF	0.29	0.150
	vWF	−0.362	0.069
	sP-sel	−0.22	0.264
LPO vs.	TF	0.243	0.252
	vWF	−0.076	0.725
	sP-sel	0.038	0.860
TV vs.	vWF	0.374	0.05
	sP-sel	−0.369	0.053
VWF vs.	sP-sel	0.467	0.012

ABPI = Ankle brachial pressure index.

Discussion

This study confirms previous observations of endothelial damage/dysfunction and abnormal platelet activation, as indicated by raised levels of vWF and sP-sel,

Fig. 1. sP-sel by time point.

Fig. 2. vWF by time point.

Fig. 3. LPO by time point.

Fig. 4. TF by time point.

Table 3. Effects of femoral cross-clamping and reperfusion

	Baseline	Just prior to clamp release	5 min after clamp release	30 min after clamp release	p value
vWF, IU/dl	139 (34)	134 (33)	133 (29)	131 (31)	0.616
sP-sel, ng/ml	49 (21)	44 (17)	44 (19)	44 (18)	0.715
TF, pg/ml	10 (10–25)	10 (10–28)	10 (10–26)	10 (10–26)	0.990
LPO, μ mol/l	8.1 (5.6–11.5)	8.0 (5.9–11.3)	7.5 (5.4–12.1)	7.5 (4.5–12.2)	0.791

Values are means (SD) or medians (interquartile range). Analysis by repeated measures ANOVA; no significant differences.

respectively [4–7, 10–13]. However, we were unable to show any significant effect of ischaemia by cross-clamping of the femoral artery during surgery and subsequent reperfusion following clamp release. This is despite a mean clamp time (and thus, ischaemia) of 28 min. Furthermore, the time frame of blood sampling was appropriate, based on previous work in this area [18, 19]. We also recognise that many of our patients had associated comorbidity, such as hypertension or diabetes, for example, which influences our research indices, but the objective of the study was to investigate the *sequential changes* following lower limb surgical revascularisation, and thus, levels would be compared to baseline levels in individual patients.

Previous experimental evidence supports the concept of ischaemic-induced endothelial damage/dysfunction. For example, in one study of endothelin (another endothelial-derived protein) in dogs, aortic cross-clamping products increased levels of endothelin suggesting possible endothelial stress [20]. Indeed, ischaemia and reperfusion were associated with disturbed cardiac and renal function and alterations in total peripheral resistance [20]. As oxygen free radicals are responsible for oxidative damage of tissues exposed to ischaemia [21], it was unsurprising to know that during balloon angioplasty of peripheral arteries, LPO, a marker of oxidative stress, was markedly increased following balloon inflation compared to baseline [14]. Another study demonstrated that peripher-

al blood showed a transient increase in markers of lipid peroxidation following successful surgery for limb salvage at intervals of >1 h after reperfusion [15]. Nevertheless, one study by Hafez et al. [16] failed to demonstrate a similar rise in patients undergoing abdominal aortic aneurysm repair.

The results of the present study concur broadly with that of Hafez et al. [16] in that there were no significant changes in the levels of LPO following cross-clamping. There is some evidence to suggest that the method of anaesthesia can attenuate the levels of LPO production in ischaemic tissues [22], and active fluid management throughout the procedure would almost certainly contribute to the reduction in the expected production of LPO. It is also possible that the sampling time points were too close together to demonstrate any significant alteration(s) in levels of the measured indices, in light of the half-lives of the indices measured. However, Lau et al. [14] did demonstrate a significant change in LPO levels, even with balloon angioplasty, which involves a shorter period of arterial occlusion.

Unfortunately we did not confirm previous observations of raised TF in patients with PVD [8], but this may be a reflection of our smaller sample size in the present study. Furthermore, we were unable to show any signifi-

cant change in plasma TF levels with ischaemia-reperfusion. Certainly, TF has been identified in several cell types associated with the atherosclerotic plaque including foam cells and monocytes [23] as well as the endothelium overlying these cells [24] suggesting that cells that are not normally thrombogenic may acquire this property, given abnormal pathophysiological processes ongoing in atherogenesis. Indeed, there was a correlation between TF and vWF, an index of endothelial damage/dysfunction in the present study, which was of borderline significance.

In conclusion, we found that during ischaemia-reperfusion during peripheral arterial bypass surgery, thrombogenesis (as measured by plasma TF) and oxidative damage (as measured by LPO) within the affected leg does not increase in the immediate perioperative period. Further studies are required to assess the mechanism(s) of ischaemia-reperfusion injury in PVD, and the contributory role(s) of the endothelium and platelets.

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