Effects of folinic acid on forearm blood flow in patients with end-stage renal disease

Todd J. Anderson¹, Yiu-Hui Sun¹, Jaroslav Hubacek¹, M. Eric Hyndman¹, Subodh Verma², Lana Shewchuk¹ and Nairne Scott-Douglas³

¹Department of Cardiovascular Sciences, Libin Cardiovascular Institute of Alberta, University of Calgary, ²Division of Cardiovascular Surgery, University of Toronto and ³Department of Medicine, Renal Division, University of Calgary, Canada

Abstract

Background. Abnormalities of endothelial function are likely to contribute to the accelerated atherosclerotic risk in subjects with end-stage renal disease (ESRD). While folates can improve endothelial function, their role in ESRD has not been fully studied. The objective was to determine the acute and 12 week-effect of folinic acid on endothelium-dependent vasodilation in subjects with ESRD.

Methods. Forearm blood flow (FBF) was assessed by strain gauge plethysmography at baseline and after 12 weeks in 34 ESRD patients (57±14 years). Vascular function was assessed with acetylcholine (ACh), and sodium nitroprusside (SNP). Patients were randomized to receive folinic acid (50 mg i.v. once weekly) or a matching placebo. A subset of 25 subjects also received folinic acid (500 μg/min intra-arterially) or placebo to determine the acute effect on ACh and SNP mediated dilation at the time of the baseline vascular study.

Results. Folinic acid acutely improved the maximum change in ACh mediated FBF (10.0±2.4 to 12.8±2.2 ml/min/100 ml, P=0.017), but did not change SNP responses. Chronic active therapy did not change ACh or SNP-mediated increases in FBF. Folinic acid resulted in a non-significant decrease in homocysteine (21±6 vs 28±18 μmol/l, P=0.16) and diastolic blood pressure was significantly reduced (P=0.05).

Conclusions. The present study demonstrated that folinic acid acutely improved endothelium-dependent vasodilatation in patients with ESRD suggesting a direct vascular effect. Chronic treatment with folinic acid did not show benefit in endothelial function, but did lower diastolic blood pressure. Further work is required to determine the optimal regime to protect vascular health in subjects with ESRD.

Keywords: atherosclerosis; dialysis; endothelium; folates; renal disease

Introduction

Atherosclerotic vascular disease is the leading cause of mortality in patients with end-stage renal disease (ESRD) including those on chronic dialysis [1]. However, this increased burden of atherogenicity cannot be fully ascribed to the clustering of coronary risk factors which are frequently present in patients with ESRD.

Hyperhomocysteinaemia has emerged as a risk factor for atherosclerosis and 90% of the patients with ESRD have elevated homocysteine levels [2]. Homocysteine has been associated with an increased mortality including individuals with renal failure. Elevated levels of homocysteine result in endothelial dysfunction, oxidative stress and lipid peroxidation, hypertension and a prothrombotic milieu [3]. While optimal treatment has not been established, folic acid and concomitant B vitamins are effective at lowering homocysteine levels in subjects with normal renal function [3]. However, lowering of homocysteine in ESRD patients is notoriously difficult. In addition, while many of the large treatment trials are nearing completion, those reported have been unable to demonstrate benefit of folic acid on cardiovascular end-points in secondary prevention and renal disease subjects [4,5].

The integrity of the endothelium regulates vascular homoeostasis through the release of factors such as endothelium-derived nitric oxide (NO). Endothelial dysfunction represents the initiating event in
atherosclerotic vascular disease and is present in subjects with hyperhomocysteinaemia and in those with ESRD. Folic acid, by lowering homocysteine levels and having favourable direct effects on NO homoeostasis has been shown to improve endothelial function [6]. Unfortunately, this strategy has not been particularly effective in improving endothelial function in adults with ESRD [7]. Methylated folates, through a more favourable interaction with NO [8] may be better suited than folic acid to augment endothelial function. However, the effect of the active forms of folic acid [folinic acid or 5-methyltetrahydrofolate (5-MTHF)] on endothelial health in subjects with ESRD has not been as well-studied [9].

The purpose of the present study was: (i) to examine the direct vascular effect of intra-arterial folic acid (methylated folic acid) on endothelium-dependent and endothelium-independent vascular function and; (ii) to determine the chronic effects of folic acid on endothelial function and homocysteine in ESRD patients receiving haemodialysis.

**Methods**

**Patient population**

Patients with greater than 3 months of haemodialysis-dependent chronic renal failure were eligible for the study and were enrolled following written informed consent. The study was approved by the Conjoint Ethics Committee of the University of Calgary. The study was carried out according to good clinical practice and the Declaration of Helsinki. The following exclusion criteria were employed: plasma homocysteine levels <15 μmol/l, patients on homocysteine lowering therapies (other than Diavite™—R&D Laboratories, Marina Del Rey, CA), patients with left ventricular dysfunction (EF <55%), uncontrolled hypertension (<170/100), unstable coronary syndromes, acute or chronic infection, changes (within one month) in endothelium-modifying medications, such as ACE-inhibitors and HMG CoA reductase inhibitors and patients who have had vascular surgery for haemodialysis access on both arms. A total of 95 subjects were approached for the study. Of these, 44 were not interested, and 51 consented. Of the consented patients, 13 were unable to participate in the study for the following reasons: six had homocysteine levels that were too low on repeat blood draw and seven had insufficient brachial artery access for the infusion studies. A total of 38 subjects were studied and complete data was available for 34 subjects.

**Assessment of endothelial function**

Forearm blood flow (FBF) responses to infused agonists were determined by a forearm mercury-in-silastic strain gauge and impedance plethysmography (Model EC-4, Hokanson Inc., Seattle, WA). Plethysmography studies were done in the morning on a day following dialysis. All the studies were performed in quiet clinical laboratory maintained at 21–23°C. All the patients fasted and refrained from drinking alcohol or caffeine-containing beverages for at least 12 h prior to the study.

The FBF was measured in the infused arm (non-shunt or AV fistula arm) during the last 3 min of each infusion. All the solutions were infused at 1.0 ml/min (Harvard Apparatus, South Natick, Massachusetts) into the brachial artery of the study arm via a 27-guage dental needle (Sherwood Medical, St Louis, Missouri) through an epidual catheter or a 20-guage IV catheter (BD Insyte-W, Becton Dickinson infusion therapy systems Inc., Sandy, Utah). During the 3 min recording, two cuffs were placed on the infusion arm and rapidly inflated by cuff inflators (model E10, Hokanson, Inc.). The cuff placed on the wrist was inflated to 20 mmHg higher than the patient’s systolic blood pressure to exclude the contribution of hand blood flow, while the cuff placed on the upper arm was inflated to 40 mmHg to occlude venous egress for 10 s of every 20 s. The plethysmographic data was continuously captured by an A–D converting board and stored in LabVIEW 5.0 software (National Instruments, Austin, TX). Measurement of blood flow in the non-infusion arm was not undertaken due to the presence of shunts or fistulas for dialysis in the majority of subjects.

The plethysmographs were calibrated to measure the percent change in volume, expressed as flow in milliliter per 100 ml tissue per minute. The slope of the volume/time relationship was calculated by LabVIEW and converted into FBF. The FBF was taken as the mean of the last five flow measurements at a given drug dose or saline infusion.

**Infusion protocol**

Endothelium-dependent and independent dilation was examined by infusion of acetylcholine (ACh) and sodium nitroprusside (SNP), respectively into the brachial artery. After successful cannulation, the following infusion scheme was employed: (i) baseline saline control (20 min), (ii) ACh, (Iolab, Claremont, California) at 3, 10 and 30 μg/min for 6 min each, (iii) recontrol saline (20–30 min), (iv) SNP, (Roche, Basel, Switzerland) at 1, 3 and 10 μg/min for 6 min each. Data was recorded for the last 3 min of each intervention period.

**Acute folic acid study**

To study the direct effects of folic acid on endothelial function, a subgroup of the study population (n = 25) was studied in the following way. After baseline infusion of ACh and SNP, a 20 min recontrol period was recorded. In a randomized and blinded fashion, folic acid (n = 20, Leucovorin Calcium, Wyeth Ayerst at 500 μg/min for 10 min) or saline (n = 5) was infused intra-arterially. The folic acid or saline was then co-infused with ACh (3, 10 and 30 μg/min) and SNP (1, 3 and 10 μg/min), respectively. The five subjects were studied using saline only to ensure blinding and to test reproducibility of repeated measures of FBF.
The entire cohort was not studied as some individuals could not tolerate the time required for the extra infusions at baseline and 25 was our a priori determined sample size to show an effect.

**Chronic folinic acid**

A total of 34 subjects (57 ± 14 years) completed the study (16 received folinic acid, 18 matching placebo). After baseline assessment of FBF, patients were randomized to receive chronic therapy with i.v. folinic acid (50 mg once per week) or matching placebo in a double-blind fashion. All subjects received pyridoxine (250 mg i.v. three times per week). The study drugs were given at the conclusion of dialysis. After 12 weeks of treatment, patients returned for repeated endothelial function testing one day following the last treatment.

**Biochemical determinations**

Biochemistry and haematology were measured by standardized assays in the laboratory at the Foothills Medical Centre, Calgary, AB. The C-reactive protein (CRP) concentrations were measured by a particle-enhanced immunoturbidimetric method with the use of a Hitachi 912 analyser (Roche Diagnostics) and reagents of Tina-quant C-reactive protein (latex) ultrasensitive assay (Roche Diagnostics). This measurement was standardized against the International Federation of Clinical Chemistry Certified Reference Material Standard (IFCC CRM 470). The lower detection limit reported for the assay was 0.21 mg/l and the coefficient of variation at 0.21 mg/l was an acceptable 7.2%. Plasma homocysteine levels were measured using a fluorescence polarization immunoassay (IMX, Abbott Laboratories, Mississauga, Ontario). The SD of the assay is 0.33 μmol/l, and the normal range for homocysteine in our laboratory is 4.9–13.7 μmol/l. The RBC folate and serum vitamin B₁₂ levels were measured using a standard commercial radioimmunoassay.

**Data analysis and statistics**

The data is expressed as mean ± SD except where indicated. Comparisons between groups were made by unpaired Student’s t-test for continuous data and chi-square for proportions, and Mann–Whitney U-test for non-parametric data. The effect of treatment on patient demographics, proportions, and Mann–Whitney U-test for non-parametric Student’s t-testing. The primary efficacy analysis for the acute folinic acid study was the change in ACh-mediated FBF with co-infusion of folinic acid compared with the preceding ACh response in the absence of folinic acid. For the chronic study, the change in ACh-mediated FBF after 12 weeks of therapy with active therapy was compared with the placebo group. This was the primary efficacy parameter and was compared by two-way repeated measures ANOVA across the dose range. The maximum change in ACh-mediated FBF was also analysed in the acute and chronic study and was assessed by paired t-testing. When the data was analysed as the percentage change from baseline, instead of absolute change, the results were the same (data not shown). The sample size was chosen to be able to detect a 30% improvement in ACh-mediated increases in FBF between the two groups. A two-sided P-value <0.05 was considered statistically significant.

**Results**

**Patient demographics**

The baseline characteristics for the overall cohort (n = 34) are summarized in Table 1. The groups were well-matched except for a trend for excess of diabetes (P = 0.10) and previous coronary disease (P = 0.17) in the placebo group. The median CRP was also higher in the folinic acid treatment group (Table 2, P = 0.025). For the 25 subjects participating in the acute study, the demographics were not different than the overall cohort and there was no difference between those who acutely received folinic acid (n = 20) or saline placebo (n = 5).

**Acute folinic acid study**

Baseline FBF was 4.3 ± 1.6 ml/min/100 g and did not change with the intra-arterial infusion of folinic acid. The ACh caused dose-dependent increases in FBF (P < 0.001). Co-infusion of folinic acid resulted in a significant increase in ACh-mediated FBF (Figure 1). The maximum change in FBF to ACh (from baseline flow) increased from 10.0 ± 2.4 ml/min/100 ml to 12.8 ± 2.2 ml/min/100 ml (P = 0.017) after folinic acid co-infusion (Figure 2). In addition, the maximum blood flow achieved with ACh increased from 14.9 ± 2.5 ml/min/100 ml to 17.9 ± 2.5 ml/min/100 ml (P = 0.005) after folinic acid co-infusion. With placebo co-infusion (n = 5), there was no change in baseline FBF, maximum change in FBF to ACh or maximum flow attained with ACh. The SNP also resulted in a dose-dependent increase in FBF (P < 0.001) that was not augmented by folinic acid. In fact, there was a trend for less maximal change with co-infusion than with SNP alone (11.1 ± 4.6 vs 13.5 ± 3.4, P = 0.08). Placebo co-infusion did not alter SNP mediated increases in FBF.

**Chronic folinic acid study**

Treatment with i.v. folinic acid resulted in marginally lower diastolic blood pressure (P = 0.05), but no

<table>
<thead>
<tr>
<th>Table 1. Patient demographics for chronic study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 18)</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>Gender (F:M)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>Height (cm)</td>
</tr>
<tr>
<td>BMI</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Hypertension</td>
</tr>
<tr>
<td>Hyperlipidaemia</td>
</tr>
<tr>
<td>Cigarette smoking</td>
</tr>
<tr>
<td>Family history</td>
</tr>
</tbody>
</table>

BMI, body mass index; CAD, presence of coronary artery disease.
changes in biochemical parameters. Homocysteine and RBC folate were not statistically different following therapy (Table 2). Homocysteine levels changed from 27.3±17.6 μmol/l to 21.6±6.1 μmol/l (P = 0.16) in the folinic acid treatment group, and from 21.6±6.1 μmol/l to 19.4±4.7 μmol/l (P = 0.20) in the placebo group. There was no change in the measured variables except for an unexplained increase in CRP in the placebo group (P < 0.01).

Basal FBF increased after 12 weeks of active therapy (4.0±1.4 vs 5.6±2.1, P = 0.02). This was not observed in the placebo group (4.4±1.6 vs 4.2±1.3). There was no significant rise in the ACh-induced increase in blood flow following active therapy (7.1±1.6 vs 8.0±0.92, P = 0.69) (Figure 3). Similarly, the difference between the ACh-mediated change (Figure 4) or maximal blood flow was not different between the active treatment and placebo groups. No effect of treatment was observed in SNP-induced FBF (Figure 5).

**Discussion**

The present study has demonstrated that folinic acid acutely improves forearm endothelium-dependent vasodilation in subjects with ESRD. However, a 12 week course of therapy failed to augment endothelial function or substantially lower homocysteine levels compared with placebo.

Through the release of a variety of autocrine and paracrine factors, particularly NO, the endothelium plays an integral role in vascular homoeostasis. Dysfunction of the endothelium is an early event in the atherosclerosis process and is seen in a variety of clinical conditions including renal dysfunction [10]. Recently, emerging evidence suggests that endothelial dysfunction is associated with adverse cardiovascular events leading to the consideration of this measure as a surrogate marker of atherosclerosis activity.

In the current study, subjects with chronic renal dysfunction on haemodialysis demonstrated endothelial dysfunction as has previously been reported [10].

---

**Table 2. Treatment effect**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Folinic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td><strong>BP–systole (mmHg)</strong></td>
<td>137±16</td>
<td>135±22</td>
</tr>
<tr>
<td><strong>BP–diastole (mmHg)</strong></td>
<td>76±10</td>
<td>75±11</td>
</tr>
<tr>
<td><strong>Homocysteine (μmol/l)</strong></td>
<td>21.8±6.5</td>
<td>19.4±4.7</td>
</tr>
<tr>
<td><strong>TC (mmol/l)</strong></td>
<td>4.04±0.87</td>
<td>3.95±0.54</td>
</tr>
<tr>
<td><strong>Triglycerides (mmol/l)</strong></td>
<td>1.78±0.77</td>
<td>1.78±0.77</td>
</tr>
<tr>
<td><strong>HDL (mmol/l)</strong></td>
<td>1.09±0.27</td>
<td>1.14±0.34</td>
</tr>
<tr>
<td><strong>LDL (mmol/l)</strong></td>
<td>2.15±0.81</td>
<td>1.99±0.48</td>
</tr>
<tr>
<td><strong>CRP (mg/l)</strong>*</td>
<td>3.7 (1.3,5.5)</td>
<td>6.9 (3.0,12.0)</td>
</tr>
<tr>
<td><strong>Glucose (mmol/l)</strong></td>
<td>8.3±4.3</td>
<td>8.2±4.0</td>
</tr>
<tr>
<td><strong>Serum B12 (pmol/l)</strong></td>
<td>497±179</td>
<td>520±279</td>
</tr>
<tr>
<td><strong>RBC folate (nmol/l)</strong></td>
<td>2284±38</td>
<td>2228±466</td>
</tr>
</tbody>
</table>

*median (quartile range); BP, blood pressure; TC, total cholesterol; CRP, C-reactive protein.

---

**Fig. 1.** Co-infusion of folinic acid acutely improves ACh-mediated increases in FBF (n = 20, *P < 0.05).

**Fig. 2.** The maximum change in FBF in response to ACh was augmented with folinic acid co-infusion (*P = 0.017), but not the SNP-mediated response in the acute intervention study.
The acute intra-arterial infusion of folinic acid resulted in the augmentation of ACh-mediated vasodilation. The lack of improvement in the SNP-mediated FBF would suggest a benefit at the level of the endothelium. To the best of our knowledge, no previous study has reported an acute effect of folates on endothelial health in subjects with ESRD. This would extend observations made with the acute administration of 5-MTHF in subjects with both familial hypercholesterolaemia and type II diabetes [11,12]. In addition, Doshi et al. [13,14] were able to demonstrate an immediate improvement in endothelial function in subjects with coronary disease following either intra-arterial 5-MTHF or oral folic acid. While traditional thought surrounding the beneficial effects of folates revolves around homocysteine lowering, the acute effect of these compounds would suggest otherwise [6]. Folates may augment NO-mediated vasodilation through anti-oxidant effects, facilitated recycling of tetrahydrobiopterin or have a direct effect on NO synthase. Studies from our group and others have suggested the potential for a direct effect of methylated folic acid on NO synthase activity [8,15]. Homocysteine certainly has direct toxic effects on endothelial cells in vitro. In addition, homocysteine produces oxygen free radicals, quenches NO and has unfavourable effects on thrombosis [3]. However, it may be that homocysteine simply reflects folate deficiency at the cellular level and is an indirect marker of vascular health.

The chronic phase of the current study did not demonstrate improvement in forearm endothelial function after 12 weeks of active therapy. Several studies in adults have shown no effect of folate supplementation on endothelial function [7,16,17]. Methylated folates have been shown to produce greater lowering of homocysteine than folic acid itself in some studies, particularly if given intravenously [18]. However, these results have not been substantiated in more recent studies [19]. More importantly, methylated folates were utilized in the present study, because of the potentially advantageous effects on NO and endothelial health [20]. In a non-randomized trial, Buccianti et al. [9] were able to demonstrate that weekly i.v. treatment with 5-MTHF for 10 weeks reduced homocysteine by 50% and improved endothelial function. We were unable to confirm a favourable long-term effect of folinic acid on forearm endothelial function in the current placebo-controlled study. Our regime utilized weekly folinic acid and three times per week pyridoxine as was done in the study by Touam et al. [18] to lower homocysteine. While there was a trend for homocysteine lowering, this was not significant with the regime used in the present trial. The RBC folate levels did not increase, but this was not surprising as saturation of this measure is known to exist at high levels as seen in the present study. However, a biological effect of active therapy was noted. The baseline FBF increased and diastolic blood pressure...
decreased after 12 weeks of therapy. This is possibly related to a decrease in peripheral vascular resistance and basal arteriolar dilation as has previously been reported. The concentration of folic acid achieved in the chronic study, although not measured, would be less than in the chronic study and might possibly account for the difference in endothelium-dependent responses. This has also been observed in previous studies of vitamin C, for example.

Clinical perspective
Atherosclerotic vascular disease is the leading cause of death in patients with ESRD [1]. Homocysteine levels are elevated in 90% of the subjects with renal failure [2]. While homocysteine is associated with an increased risk in subjects with both normal renal function and renal failure, the utility or best approach to lower homocysteine has not been established. Wrone et al. [5] were unable to demonstrate a beneficial effect of folic acid up to 15 mg daily on cardiovascular endpoints in subjects with ESRD. Ongoing trials will establish the role of folates in other clinical entities, but a recently reported study did not show the benefit of folic acid following stroke [4]. Direct vascular effects of the active form of folic acid were demonstrated in the present study, however lasting effects could not be maintained. No evidence is presented here to suggest that a more aggressive treatment of the folate/homocysteine axis than the current approach of low dose B vitamins is warranted. Other strategies to lower traditional cardiovascular risk factors in subjects with ESRD may be more fruitful, but this requires further study. However, the lack of benefit from atoravastatin in renal failure subjects in a recently published trial might suggest the difficulty of single intervention in this patient population.

Limitations
The sample size was chosen to detect a 30% difference in endothelium-dependent vasodilation between the two groups. Smaller changes could be missed. The regimen utilized did not significantly lower homocysteine levels in the active group. A strong trend was observed, however. As such, while we can reasonably exclude a chronic direct vascular effect of folic acid on endothelial health at the dose studied, we cannot completely exclude a homocysteine mediated effect. Further studies with regimens that normalize homocysteine levels will be required. The mechanism of the acute improvement in ACh-mediated endothelial function cannot be assessed with this type of study.

Conclusion
The present study demonstrated that the methylated folate derivative, folic acid acutely improved endothelium-dependent vasodilatation in patients with ESRD suggesting a direct vascular effect independent of changes in homocysteine level. Chronic treatment with folic acid in ESRD patients did not show benefit in endothelial function or homocysteine levels. Further work is required to determine the optimal regime to protect vascular health in subjects with ESRD.

References
11. van Etten RW, de Koning EJ, Verhaar MC, Gaillard CA, Rabelink TJ. Impaired NO-dependent vasodilatation in patients with Type II (non-insulin-dependent) diabetes mellitus is restored by acute administration of folate. Diabetologia 2002; 45: 1004-1010
12. Verhaar MC, Wever RMF, Kastelein JJP et al. 5-Methyltetrahydrofolate, the active form of folic acid restores

Received for publication: 31.1.06
Accepted in revised form: 7.2.06