The Effect of Folate and Vitamin B6 on Endothelial Function in Children with Type 1 Diabetes

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Abstract

Introduction

Endothelial dysfunction is a precursor of vascular disease. Children at high risk of vascular disease including children with type 1 diabetes (T1DM) have marked endothelial dysfunction. Endothelial dysfunction is reversible occurring early in the time-line of atherosclerosis. The detection of endothelial dysfunction in childhood allows the study of interventions at an early and potentially reversible stage of vascular damage.

We have previously shown that endothelial dysfunction is common in children with T1DM and relates to folate status (Wiltshire, Gent *et al*. 2002) despite higher serum and red cell folate levels and lower total plasma homocyst(e)ine (tHcy) than healthy controls (Wiltshire, Thomas *et al*. 2001; Wiltshire and Couper 2004). Even with these higher folate levels, in a pilot, cross-over study we have shown that folate supplementation improves endothelial function in children with T1DM (Pena, Wiltshire *et al*. 2004).

Beneficial effects of folate on endothelial function are being demonstrated in increasing numbers of studies (Verhaar, Wever *et al*. 1998; Woo, Chook *et al*. 1999; Doshi, McDowell *et al*. 2001; Thambyrajah, Landray *et al*. 2001; van Etten, de Koning *et al*. 2002; Woo, Chook *et al*. 2002). Improvement in endothelial function, has also been observed within hours of additional oral folate (Doshi, McDowell *et al*. 2002) and within minutes of intravenous 5-methyltetrahydrofolate (MTHF), the active form of folate (Verhaar, Wever *et al*. 1998; van Etten, de Koning *et al*. 2002).

Treatment with combination folate and vitamin B6 lowers markers of endothelial activation (Constans, Blann *et al*. 1999; Vermeulen, Stehouwer *et al*. 2000). However, there is limited literature examining the effect of B6 alone on the endothelium. Vitamin B6 improves endothelial function in cardiac transplant recipients (Miner, Cole *et al*. 2001). There is no data examining the effect of supplemental vitamin B6 in T1DM or children at risk of vascular disease.

Atherosclerosis is an inflammatory process and high-sensitivity C-reactive protein (Hs-CRP), a marker of inflammation, predicts cardiovascular events in adults. Elevated Hs-

CRP in otherwise healthy children is associated with impaired endothelial function. Similar studies in children with T1DM have not been performed.

We therefore aimed to determine the effects, acutely, of folate and vitamin B6 on endothelial function, and over eight weeks, of folate and vitamin B6, alone and in combination, on endothelial function. In addition, we sought to determine whether Hs-CRP, is associated with vascular endothelial and smooth muscle dysfunction, in children with T1DM and healthy control subjects.

Methods

A randomised, double-blind, placebo-controlled study of folate 5mg daily and vitamin B6 100mg daily in 124 children with T1DM determined the immediate and eight week effects of these vitamins, alone and in combination, on endothelial function. Endothelial function, assessed by flow mediated dilatation(FMD) and glyceryl-trinitrate(GTN)-induced dilatation using high resolution ultrasound of the brachial artery, was measured at baseline, at two and four hours after the first dose (n=35), and at four and eight weeks of treatment (n=122). Serum and red cell folate, serum vitamin B6, Hs-CRP, tHcy, HbA1c and blood glucose were measured at each assessment of endothelial function.

Hs-CRP and endothelial function, were measured at baseline, in 121 subjects with T1DM. 31 subjects with T1DM that were randomised to receive placebo treatment were studied at four and eight weeks and were included in the longitudinal analysis of Hs-CRP and endothelial function. Hs-CRP and endothelial function were also studied in 33 age-matched, healthy control subjects.

Results

FMD normalised in all treatment groups. At baseline and eight weeks FMD [mean(SD)] on folate improved from 2.6(4.3)% to 9.7(6.0)%($p<0.001$), on vitamin B6 from 3.5(4.0)% to 8.3(4.2)%($p<0.001$), and on folate/vitamin B6 from 2.8(3.5)% to 10.5(4.4)%($p<0.001$) respectively. This improvement in FMD occurred within two hours and was maintained

over eight weeks for each treatment. FMD in the placebo group, and GTN-induced dilatation in all groups, did not change. Increase in serum folate, red cell folate, and vitamin B6 related to increase in FMD. Improvement in FMD was independent of change in tHcy, glucose, HbA1c and Hs-CRP. Baseline red cell folate and baseline diastolic blood pressure inversely related to improvement in FMD. Serum triglycerides and LDL-cholesterol inversely related to baseline FMD.

Hs-CRP did not differ between subjects with T1DM and healthy, age-matched controls. In both controls and subjects with T1DM, Hs-CRP did not relate to FMD or GTN-induced dilatation at baseline or at intervals over eight weeks in subjects with T1DM. Hs-CRP did not change over time. In T1DM, but not healthy controls, Hs-CRP related to BMI z-score($r=0.47, p<0.001$), weight z-score($r=0.41, p<0.001$) and female sex($p=0.008$).

Conclusions

High dose folate and vitamin B6 rapidly normalise endothelial dysfunction in children with T1DM. This effect is maintained over eight weeks with ongoing supplementation. Combination treatment over eight weeks does not confer additional benefit.

Hs-CRP is not associated with early vascular dysfunction in children with T1DM. However, in children and adolescents with T1DM, Hs-CRP is associated with female sex and children with higher BMI suggesting these groups may be at greater cardiovascular risk.

In addition to optimising metabolic control, intervention with folate or vitamin B6, at an early stage in childhood, could have a major impact on long-term diabetic vascular complications, and requires further investigation. Maintenance of a healthy BMI may be important in the prevention of vascular disease of T1DM.

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Abbreviations

2D	Two-dimensional
ACE	Angiotensin converting enzyme
AER	Albumin excretion rate
AGEs	Advanced glycation end products
AST	Aspartate aminotransferase
BH_2	Dihydrobiopterin
BH_4	Tetrahydrobiopterin
cGMP	cyclic 3’5’ guanosine monophosphate
CHD	Coronary heart disease
CRP	C-reactive protein
CYWHS	Children, Youth and Women’s Health Service
DAG	Diacylglycerol
DCCT	Diabetes Control and Complications Trial
DKA	Diabetic ketoacidosis
ECG	Electrocardiogram
EDIC	Epidemiology of Diabetes Interventions and Complications
EDRF	Endothelium derived relaxing factor
EDTA	Ethylene-diamine-tetra acetic acid
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FDA	Food and Drug Administration
FMD	Flow mediated dilatation
GFR	Glomerular filtration rate
GTN	Glyceryl trinitrate
HbA1c	Haemoglobin A1c
HDL	High density lipoprotein
Hs-CRP	High sensitivity C- reactive protein
IDDM	Insulin dependent diabetes mellitus
IDF	International Diabetes Federation
Ig	Immunoglobulin
IMT	Intimal medial thickness
IMVS	Institute of Medical and Veterinary Science
LDL	Low density lipoprotein

LED	Light emitting diode
MTHF	Methyltetrahydrofolate
MTHFR	Methylene tetrahydrofolate reductase
NADPH	Nicotinamide Adenine Dinuleotide Phosphate (reduced)
NF-κB	Nuclear factor kappa B
NO	Nitric oxide
ORPS	Oxford Regional Prospective Study
PAI-1	Plasminogen activator inhibitor-1
PGA	Pteroylmonoglutamate
PKC	Protein kinase C
PLP	Pyridoxal 5'-phosphate
RAGE	Receptors for AGE
RCT	Randomised Controlled Trial
ROS	Reactive oxygen species
SAH	S-adenosyl-L-homocysteine
T1DM	Type 1 Diabetes Melllitus
TGF- β	Transforming growth factor-β
tHcy	Total plasma homocyst(e)ine
TSH	Thyroid stimulating hormone
U.S.	United States
VEGF	Vascular endothelial growth factor
VD	Vessel diameter
VLDL	Very low density lipoprotein
vWF	von Willebrand Factor
WCH	Women's and Children's Hospital

Units

µg/min	micrograms per minute
µmol/l	micromoles per litre
µg	micrograms
µg/l	micrograms per litre
℃	degrees Celsius
cm	centimetres
kg	kilogram
kg/m^2	kiligograms per square metre
m/s	metres per second
mg/day	milligrams per day
mg/l	milligram per litre
mm	millimetre
mmHg	millimeters mercury
mmol/l	millimoles per litre
nmol/l	nanomoles per litre
units/kg	units per kilogram