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SHORT COMMUNICATION

Riboflavin status in acute ischaemic stroke

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Background: There is experimental evidence that riboflavin (vitamin B2) supplementation reduces oxidative damage and cerebral oedema following acute stroke.
Objective: To measure riboflavin levels in acute stroke before and after supplementation with this vitamin.
Design: Ninety-six acute ischaemic stroke patients had their riboflavin status measured at baseline and then randomly assigned to receive 5 mg of oral riboflavin and other B-group vitamins within 12 h of the stroke onset and then daily or no B-vitamins for 14 days. Non-fasting venous blood was obtained at baseline, days 7 and 14 post-randomization for measurement of riboflavin status using erythrocyte glutathione reductase activity coefficient (EGRAC). EGRAC is a measure of riboflavin tissue saturation. This assay has the advantage of being extremely stable and sensitive. EGRAC values are inversely proportional to riboflavin status, so that values greater than 1.3 indicate biochemical deficiency.
Results: Fifty-one per cent of patients studied were riboflavin deficient at baseline. Fourteen days of riboflavin supplementation significantly improved the measure of B2 status compared with the control group. Seven out of 37 patients in the supplement group (19%) were riboflavin deficient compared with 22 out of 39 patients (56%) in the control group at the end of the treatment period ($P = 0.035$ for the differences in cumulative changes between groups over 2 weeks).
Conclusions: A high proportion of acute stroke patients were biochemically deficient of riboflavin immediately post-infarct. Supplementation with 5 mg of riboflavin for 2 weeks significantly improved riboflavin status; however, the clinical significance of these findings is not yet known.
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Keywords: ischaemia; stroke; riboflavin

Introduction

Worldwide stroke is a common and devastating event with immense human and financial costs. Epidemiological and experimental evidence suggest a probable role for certain B-group vitamins in the pathogenesis of stroke (Hankey and Eikelboom, 1999). For example, recent meta-analyses predict that lowering homocysteine would significantly reduce the risk of stroke (Homocysteine Studies Collaboration, 2002). Increased intakes of B-vitamins have, via their cofactor roles in homocysteine metabolism, homocysteine-lowering effects (Hankey and Eikelboom, 1999). A recent study has shown for the first time a marked homocysteine-lowering effect of riboflavin supplementation in individuals with methylenetetrahydrofolate reductase (MTHFR) 677 TT genotype (McNulty *et al*., 2006).

Riboflavin is thought to protect tissues from ischaemia/reperfusion injury, probably through a redox effect (Hultquist *et al*., 1993; Mack *et al*., 1995). In rats exposed to focal cerebral ischaemia pre-treatment with riboflavin decreased cerebral oedema (Betz *et al*., 1994). It was suggested in this and other studies that riboflavin reacts with oxidized iron to reduce oxidative injury and cell death. If riboflavin is protective in this way then low riboflavin status, for which there is some evidence in healthy free-living older people (Finch *et al*., 1998), might imply that there is less protection from oxidative injury in ischaemic stroke. Supplementing riboflavin during oxidative stress, therefore, would appear to be an attractive proposition. We have recently reported that B-group vitamin supplementation with or without antioxidants immediately post-infarct may have antioxidant and

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anti-inflammatory effects in stroke disease independent of their homocysteine-lowering effect (Ullegaddi *et al*., 2006). The aim of this report is to present some preliminary baseline data on riboflavin status in ischaemic stroke patients and after supplementation with this vitamin.

Methods

Details of the methodology of this paper have been published before (Ullegaddi *et al*., 2006). In brief, after an informed written consent, 96 acute ischaemic stroke patients admitted to a Teaching hospital, UK, within 12 h of symptom onset were randomly assigned to receive oral supplements of 5 mg vitamin B2 and other B-group vitamins including 5 mg folic acid, 50 mg vitamin B_6 and 0.4 mg of vitamin B_{12} ($n=48$) daily, or no B-group vitamins ($n=48$) for 14 days. Stroke patients with active gastrointestinal disease, severe medical or psychiatric illness, serum creatinine concentration $>150\,\mu$mol/l, history of gout or acute renal failure, supplemental vitamins, or inability or refusal to give consent were excluded. Treatment group and controls were matched for stroke subtype and age (<75, $\geqslant 75$ years). The classification of stroke subtypes was based on clinically identifiable subtypes of cerebral infarction as described by Bamford *et al*. (1991). This classification has important prognostic implications and is useful for planning stroke treatment trials.

Protocol

Non-fasting venous blood was obtained before treatment (within 12 h of stroke onset) and at days 7 and 14. Following separation from red blood cells, plasma was stored, with stabilizer where appropriate, at -70°C for analysis within 12 weeks. Red blood cells were washed, haemolysed and stored at -70°C for the later measurement of vitamin B2 status. All analyses were performed blind to the identity of the sample. Riboflavin status was assessed as the erythrocyte glutathione reductase activity coefficient (EGRAC), using the Cobas BioAutoanalyser (Roche Diagnostics, Indianapolis, IN, USA) (Powers *et al*., 1983) giving an interbatch coefficient of variation (CV) of 3.4%. Ratios above 1.3 were considered to reflect biochemically deficiency of riboflavin. All patients had demographic and medical data collected at baseline. Repeated measures analysis of variance test was used to test within and between-subject differences and P-value < 0.05 was considered significant.

The study was approved by the Local Health Research Ethics Committee, and all subjects gave informed written consent.

Results

Table 1 shows baseline characteristics of treatment group and controls. The two groups were comparable on entry into the study with respect to age, clinical stroke sub-type, drug and alcohol intake, chronic diseases and time between stroke onset and randomization. Although there were differences in some baseline characteristics such as prevalence of atrial fibrillation, diabetes, blood glucose and serum creatinine these differences did not reach statistical significance.

Vitamin supplements were prescribed in patients' drug cards and compliance for surviving patients was 100% with no treatment failure or withdrawal. Forty-nine out of 96 stroke patients (51%) were riboflavin deficient at baseline (Figure 1). Fourteen days of riboflavin supplementation significantly improved the measure of B2 status compared with the control group with only seven out of 37 patients (19%) completed the study were riboflavin deficient in the supplement group compared with 22 out of 39 patients (56%) in the control group at the end of the treatment period ($P=0.035$ for the differences in cumulative changes between groups (Table 2)).

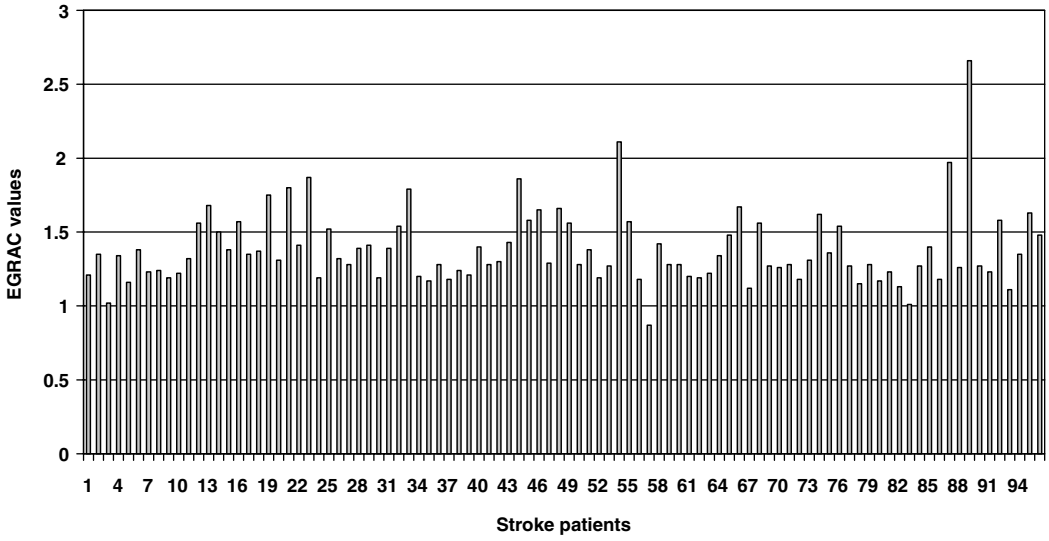
Table 1 Baseline data for the vitamin B2 and control group

	*B2-vitamin (EGRAC) (n = 48)**	*Control (n = 48)**
Median age (inter-quartile range)	76 (67–81)	77.5 (70–84)
Sex, males (%)	27 (56)	24 (50)
Stroke sub-type (%)		
TAC1	12 (25)	12 (25)
PAC1	16 (33)	16 (33)
LAC1	12 (25)	12 (25)
POC1	8 (17)	8 (17)
Smoking history (%)		
Never smoked	10 (42)	12 (50)
Ex-smoker (>1 month)	11 (46)	8 (33)
Current smoker	3 (12)	4 (17)
Alcohol intake >21 U/week (%)	2 (4)	3 (6)
Drugs[a] per patient (inter-quartile range)	1.9 (1.9–2.0)	1.5 (1.5–2)
Previous stroke/TIA (%)	18 (38)	18 (38)
AF on ECG (%)	7 (15)	11 (23)
Hypertension (%)	15 (31)	18 (38)
Ischaemic heart disease (%)	9 (19)	12 (25)
Diabetes mellitus (%)	6 (13)	1 (2)
Median Barthel score (inter-quartile range)	48 (20–74)	50 (21–70)
Median blood glucose, mmol/l (inter-quartile range)	5.8 (5.4–7.4)	6.4 (5.6–7.4)
Median serum creatinine, μmol/l (inter-quartile range)	84 (68–104)	79 (64–114)
Median hours from onset of stroke to randomization (inter-quartile range)	10 (5.0–13)	9.5 (5.0–12.0)

Abbreviations: AF, atrial fibrillation; ECG, electrocardiogram; EGRAC, erythrocyte glutathione reductase activity coefficient; LAC1, lacunar; PAC1, partial anterior circulation; POC1, posterior circulation; TAC1, total anterior circulation; TIA, transient ischaemic attack.

[a]Diuretics, ACE inhibitors, β-blockers, calcium channel blockers, statins, aspirin warfarin, clopidogrel.

*No statistically significant difference between the two groups in any of the baseline variables.



EGRAC values greater than 1.3 indicate riboflavin biochemical deficiency.

Figure 1 Baseline riboflavin status in stroke.

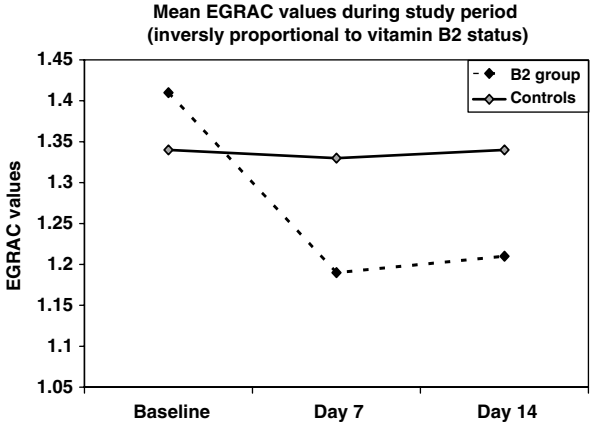
Table 2 Repeated measures ANOVA for the differences in cumulative changes between the riboflavin (B2) supplement group and controls tests of between-subjects effects

Source	*Type III sum of squares*	*Degrees of* freedom	*Mean square*	F-*values*	P-*value*
Intercept	377.289	1	377.289	7489.550	0.000
B2 supplement vs control	0.232	1	0.232	4.613	0.035

Abbreviation: ANOVA, analysis of variance.

Figure 2 shows significant reduction in EGRAC (inversely associated with vitamin B2 status) in the intervention groups compared with controls, after adjusting for smoking, drug intake, chronic diseases and inflammation.

We found no correlation between EGRAC and clinical stroke subtype, tissue inflammation, previous illness, smoking or medications.



	Baseline	Day 7	Day 14
B2 group (n=48)	1.41 (0.24)	1.19 (0.12)	1.21 (0.10)
Controls (n=48)	1.36 (0.26)	1.33 (0.17)	1.34 (0.16)

p=0.035 for the differences in cumulative changes between groups at day 14

Figure 2 Mean EGRAC concentration during study period.

Discussion

A high proportion of stroke patients studied were biochemically deficient of riboflavin at baseline. Supplementation with 5 mg of riboflavin immediately post-infarct significantly improved riboflavin status in stroke patients. Although our sample size was small and larger studies need to be undertaken to determine the biological and functional relevance of these findings, preliminary experimental studies demonstrated that, administration of riboflavin protect brain tissue from oxidative ischaemic damage and prevent oedema formation (Mack *et al*., 1995). A recent study in rats has shown that administration of riboflavin improves behavioural outcome and reduces oedema formation and glial fibrillary acidic protein expression after traumatic brain injury (Hoane *et al*., 2005). McNulty *et al*. (2006) have recently reported for the first time a marked homocysteine-lowering effect of riboflavin supplementation specifically in individuals with MTHFR 677 TT genotype. They also suggested that their findings might have implications for public health policies aimed at reducing the burden of cardiovascular diseases in Europe but not in North America, where riboflavin fortification has existed for long time. This argument is further strengthened by the finding of the recent National Diet and Nutrition Survey of older persons,

which identified low biochemical status of riboflavin in 40% of the older population in the UK (Finch *et al*., 1998).

Although vitamin B2 was used with other B-group vitamins in this study we found no evidence of interactions (Ullegaddi *et al*., 2006). Also we cannot exclude the effects of differences in rate of absorption and/or dietary intakes between subjects; however, if present, the effect of the latter is likely to be modest given the dose of vitamin B2 used.

In conclusion, we found a high proportion of acute stroke patients were biochemically deficient of riboflavin immediately post-infarct and that supplementation with this vitamin significantly improved riboflavin status; however, the clinical significance of these findings needs further investigation.

Acknowledgements

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