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Double-blind randomised controlled trial of folate treatment before conception to prevent recurrence of neural-tube defects

K M LAURENCE, NANSI JAMES, MARY H MILLER, G B TENNANT, H CAMPBELL

Abstract

A randomised controlled double-blind trial was undertaken in south Wales to prevent the recurrence of neural-tube defects in women who had had one child with a neural-tube defect. Sixty women were allocated before conception to take 4 mg of folic acid a day before and during early pregnancy and 44 complied with these instructions. Fifty-one women were allocated to placebo treatment. There were no recurrences among the compliant mothers but two among the non-compliers and four among the women in the placebo group. Thus there were no recurrences among those who received supplementation and six among those who did not; this difference is significant ($p = 0.04$).

It is concluded that folic acid supplementation might be a cheap, safe, and effective method of primary prevention of neural-tube defects but that this must be confirmed in a large, multicentre trial.

Introduction

Despite the recent fall in the incidence of neural-tube defects[1] and despite prenatal screening for α-fetoprotein, amniocentesis, and selective abortion, neural-tube defects are still the most common serious malformation found in the UK at birth,[2] affecting women from social classes IV and V more often than those from social classes I and II.[3 4] Mean blood concentrations of various essential substances, including vitamins and folic acid, are lower in the lower social classes, and are lower still in women pregnant with a child with a neural-tube defect.[5] A pilot study by one of us (KML) also suggested that these women had low concentrations of red-cell folate. More specifically, Hibbard and Smithells suggested that folic acid metabolism as measured by the FIGLU excretion test is more often deranged in the puerperium in women who have just given birth to a child with a neural-tube defect as compared with controls.[6] Some of these findings, together with a report that aminopterin, a powerful folic acid antagonist, used for a short time to procure therapeutic abortion caused several cases of anencephaly,[7] prompted us to investigate the role of folic acid in early pregnancy and the effect of folic acid supplementation before conception on the prevention of neural-tube defects.

Subjects and methods

Women resident in Glamorgan and Gwent who had had a pregnancy complicated by a fetal neural-tube defect (anencephaly, encephalocele, and spina bifida cystica) between 1954 and 1969 were traced through malformation registers, maternal and paediatric records, local authority records, and other sources. Those under 35 years of age at the time of the study were visited in their homes by medically qualified fieldworkers. NJ visited 479 mothers in West Glamorgan and the western part of Mid Glamorgan (area A), and MM visited 426 in the eastern part of Mid Glamorgan, South Glamorgan, and the western portion of Gwent (area B). These two areas were studied by Laurence *et al*[8] from 1956 to 1962 and by Richards[9] in 1964-6 and had similar incidences of neural-tube defects—namely, about 7·0 per 1000 singleton births.[4 9]

During the home visit a questionnaire was completed giving details of the woman's diet during the interpregnancy period and during her previous pregnancies. A simple diet sheet was used that provided a general pattern for meals and a check list showing the amount of food consumed during the average week and listing first-class proteins, dairy products, fresh vegetables and salads, cereals, and refined

Department of Child Health, Welsh National School of Medicine, Cardiff CF4 4XN

K M LAURENCE, DSC, FRCP(E), professor of paediatric research and clinical geneticist
NANSI JAMES, MB, MRCP, fieldworker
MARY H MILLER, MB, DCH, fieldworker

Department of Haematology, Welsh National School of Medicine

G B TENNANT, MSC, senior scientific officer

Department of Medical Statistics, Welsh National School of Medicine

H CAMPBELL, FRCP, FRSS, professor

carbohydrates, paying special attention to those items rich in folic acid. Diets were judged as good, fair, or inadequate, those that were poor or fair but deranged by an excessive amount of fat and refined carbohydrates being judged as inadequate.[10] A sample of blood was taken from all women who were planning to have further children for estimation of serum and red-cell folic acid concentrations by a modified lactobacillus method[11 12] and for other laboratory investigations. Those willing to co-operate were asked to take twice a day a tablet containing either 2 mg of folic acid or placebo starting from the time contraceptive precautions were stopped. Women were allocated to receive treatment or placebo by random numbers and did not know the content of the tablets; we were also unaware of the treatment prescribed. Women were instructed to report to us within six weeks of a missed period and were revisited as soon as possible thereafter. Inquiries were made about the quality of the diet during the current pregnancy and about any anorexia or vomiting, drugs, or illness, and a further sample was taken for folate estimation and other investigations. The women were revisited at six months and again at the end of pregnancy, when details of the outcome of the pregnancy were available.

Results

Altogether 905 women who had had a child with a neural-tube defect were seen by the fieldworkers, of whom 111 (12·3%) agreed to take part in the prophylactic randomised controlled trial and achieved a subsequent pregnancy. Of these, 60 had been randomised to receive folate supplementation and 51 placebo.

Compliance in taking the folate tablets was monitored at the sixth to ninth week of estimated gestation; if the serum folate concentration at this stage was higher than 10 μg/l the woman's account of taking the tablets during the earlier part of the pregnancy could be accepted as valid. If the serum folate concentration was below 10 μg/l the woman was classified as a non-complier. None of the placebo group had a serum folate concentration above 12 μg/l. One woman had an exceptionally high serum folate concentration (212 μg/l, which was over 10 SDs above the mean) with a normal red-cell folate concentration. On further investigation at the end of the study she admitted that she had not taken the folate tablets during early pregnancy but had taken a large number of them at seven weeks' gestation, just before the fieldworker was due to visit her. She was also classified as a non-complier. Her pregnancy terminated at three months in a spontaneous abortion of an anencephalic fetus. Including this case, there were 16 non-compliers (27%) among the 60 women allocated to receive folate treatment (table I). Compliance was not tested among the controls.

Six pregnancies resulted in a fetus with a neural-tube defect (table I): none in the compliers, two in the non-compliers, and four in the placebo group. Table II gives details of these six recurrences.

Table III shows the numbers of women taking a good, fair, or inadequate diet classified by outcome of pregnancy and whether they had received folate treatment. The proportion of women with inadequate diets was similar in the two treatment groups: 10 out of the 44 compliers and 17 out of the 67 non-compliers and women in the placebo group. All six of the recurrences of fetal neural-tube defects occurred in women taking an inadequate diet.

Table IV shows the mean red-cell folate concentration in each treatment group by the adequacy of the diet. In each dietary group the compliers had a mean concentration at least twice that in the placebo group, and these differences were significantly different ($p < 0·001$); but the concentrations in the non-compliers were similar to those in the placebo group. Within the compliers there were no significant differences between the dietary groups, but within the two untreated groups the women whose diets were poor had a reduced red-cell folate concentration. The mean concentration in the six women with a recurrence of fetal neural-tube defects was 249 μg/l, which, although lower than the mean in the untreated women, was not significantly different from it. There was no particular pattern for the mean serum folate concentrations between the dietary groups.

TABLE I—*Outcome of pregnancy by treatment group*

Outcome of pregnancy	Folate groups		Placebo group
	Compliers	Non-compliers	
Normal fetus	44	14	47
Fetus with neural-tube defect	0	2	4
All cases	44	16	51

TABLE III—*Numbers of women taking good, fair, or inadequate diets classified according to whether they received folate treatment and whether fetus was normal or had a neural-tube defect*

Diet	Received folate		Did not receive folate		All cases
	Normal	Neural-tube defect	Normal	Neural-tube defect	
Good	17	0	26	0	43
Fair	17	0	24	0	41
Inadequate	10	0	11	6	27
All cases	44	0	61	6	111

TABLE IV—*Mean ± SD red-cell folate concentration (μg/l red blood cells) by treatment group and quality of diet*

Diet	Folate groups		Placebo group
	Compliers	Non-compliers	
Good	618 ± 60 (n = 17)	277 ± 44 (n = 5)	278 ± 25 (n = 21)
Fair	847 ± 60 (n = 17)	292 ± 23 (n = 6)	298 ± 34 (n = 18)
Inadequate	761 ± 85 (n = 10)	193 ± 34 (n = 5)	250 ± 26 (n = 12)
All cases	738 ± 42 (n = 44)	256 ± 22 (n = 16)	278 ± 16 (n = 51)

Discussion

The first objective of the study was to recruit enough women who had had a child with a neural-tube defect to take part in a randomised controlled trial; in this we were only partially successful because after five years and interviewing 905 women we had recruited 111 (12·3%) women who agreed to take part and achieved a subsequent pregnancy. Moreover, of the 60 women allocated to receive folate treatment, 16 (27%) did not comply with instructions to take the tablets.

As a trial of the methodology of preventing neural-tube defects by giving prophylactic folate the trial was unsuccessful: two out of 60 women allocated to receive treatment had a recurrence compared with four out of 51 controls. This disappointing result was due to non-compliance with the tablet regimen. As a trial of the biological effects of receiving folate during early pregnancy, however, the study was more successful.

TABLE II—*Folate concentrations and outcome of pregnancy in the six women whose pregnancies were complicated by fetal neural-tube defects*

Case No	Treatment	Diet	Blood estimations			Outcome		
			Gestational age (weeks)	Serum folate (μg/l)	Red-cell folate (μg/l)	Delivery	Lesion	Gestational age (months)
1	Folate non-complier	Poor	7	212·0	259	Miscarriage	Anencephaly	3
2	,,	,,	8	4·8	155	Live birth	Spina bifida cystica	9
3	Placebo	,,	8	3·7	228	Live birth	Spina bifida cystica	8
4	,,	,,	7	11·0	275	Miscarriage	Anencephaly	4
5	,,	,,	8	5·0	380	Live birth	Spina bifida cystica	9
6	,,	,,	7	2·9	70	Termination	Anencephaly	5

None of the 44 women who received treatment had a recurrence, whereas there were six recurrences among the 67 untreated cases. The probability of such a distribution, using Fisher's exact test with a single tail, was $p = 0.04$.

The specific effect of folate has to be separated from the non-specific effect of diet. There were no recurrences among the 84 women who received good or fair diets, but there were six recurrences among the 27 women receiving a poor diet ($p < 0.0001$, Fisher's exact test). As we have shown,[11] women who take poor diets are at an extremely high risk of a recurrence of fetal neural-tube defects. Within this high-risk group of women, however, there were no recurrences in the 10 who had taken folate supplementation but six recurrences in the 17 who had not taken supplementation ($p = 0.04$, Fisher's exact test). Thus although there may have been some bias owing to women who were receiving an inadequate diet also failing to comply, yet within this group receiving an inadequate diet the preventive effect could still be detected. We conclude that women receiving a poor diet who are at high risk of a recurrence of fetal neural-tube defects can reduce their risk either by improving their diet or by taking folate supplements.

The use of folate as an effective prophylactic regimen to prevent neural-tube defects in high-risk groups, communities with a high incidence of such defects, or even all women at risk of pregnancy should be further tested in a larger controlled trial conducted at several centres. This present study shows that there would be difficulties in recruitment and in ensuring compliance; consequently such a trial would require skilled fieldworkers and the compliance would have to be verified by laboratory estimations of either folate concentrations or other biochemical markers. This would necessarily imply the expense of fieldworkers and technicians. Such a trial would be justified scientifically because there are sound reasons to suggest that once the chorioallantoic placenta is formed and the fetal heart starts to perfuse it (about 22 days after fertilisation in man) the folate requirements of the conceptus increase steeply. Deficiency or unavailability of folate at this stage might, therefore, interfere with the orderly closure of the neural tube (F Beck, personal communication).

Such a trial would be ethical as we found a probably biological beneficial effect, but the problem might be to consider an alternative regimen. A placebo could be justified by the argument that it is not normal practice to begin supplementation before conception is confirmed. As a result of the study by Smithells *et al*[13] Pregnative Forte F without folate would seem to be a suitable alternative. With an expensive blunderbuss preparation of that type, which includes several agents in addition to folate, the specific beneficial agent and the hazards that might arise from the other constituents should be identified. With folate the only hazard might be the risk of subacute combined degeneration of the cord in the presence of vitamin B_{12} deficiency. This must be very rare, but perhaps in women over 35 years B_{12} concentrations could be measured on one occasion. Otherwise folate treatment has no known hazards, is fairly cheap, is easily taken, and if effective as a primary preventive measure in planned pregnancy would greatly reduce the costs and hazards of screening for α-fetoprotein, amniocentesis, and termination of pregnancies and the cost of medical and custodial care of live-born infants with neural-tube defects.

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References

1 Bradshaw J, Weale J, Weatherall J. Congenital malformation of the central nervous system. *Population Trends* 1980;**19**:13-18.

2 Laurence KM. Prevention and prenatal diagnosis of neural tube defects. In: Persaud TV, ed. *Advances in the study of birth defects*. Vol 7. Lancaster: MTS Press (in press).

3 Record RG, McKeown T. Congenital malformations of the central nervous system. I. Survey of 930 cases. *British Journal of Preventive and Social Medicine* 1949;**3**:183-219.

4 Laurence KM, Carter CO, David PA. The major central nervous system malformations in South Wales. II. Pregnancy factors, seasonal variations and social class effects. *British Journal of Preventive and Social Medicine* 1968;**22**:212-22.

5 Smithells RW, Sheppard S, Schorah CJ. Nutritional deficiencies and neural tube defects. *Arch Dis Child* 1976;**51**:944-50.

6 Hibbard ED, Smithells RW. Folic acid metabolism and human embryopathy. *Lancet* 1965;i:1254.

7 Thiersch JB. Therapeutic abortions with folic acid antagonist, 4-aminopteroylglutamic acid (4 amino PGA) administered by oral route. *Am J Obstet Gynecol* 1952;**63**:1298-1304.

8 Laurence KM, Carter CO, David PA. The major central nervous system malformations in South Wales. I. Incidence, local variation and geographical factors. *British Journal of Preventive and Social Medicine* 1968;**22**:146-60.

9 Richards IDG. *Congenital defects in South Wales*. PhD thesis. University of Wales, 1971.

10 Laurence KM, James N, Miller M, Campbell H. The increased risk of recurrence of neural tube defects to mothers on poor diets and the possible benefit of dietary counselling. *Br Med J* 1980;**281**:1542-4.

11 Tennant GB, Withey JL. An assessment of work simplified procedures for the microbiological array of serum vitamin B12 and serum folate. *Medical Laboratory Technology* 1972;**29**:171-81.

12 Hoffbrand AV, Newcombe BFA, Mollin DL. Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. *J Clin Pathol* 1966;**19**:17-28.

13 Smithells RW, Sheppard S, Schorah CJ, *et al*. Possible prevention of neural tube defects by preconceptional vitamin supplementation. *Lancet* 1980;i:339-40.

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BRANK URSINE. Besides the common name Brank-Ursine, it is also called Bear's-breach, and Acanthus, though I think our English names to be more proper; for the Greek word *Acanthus*, signifies any thistle whatsoever.

This thistle shoots forth very many large, thick, sad green smooth leaves on the ground, with a very thick and juicy middle rib; the leaves are parted with sundry deep gashes on the edges; the leaves remain a long time, before any stalk appears, afterwards rising up a reasonable big stalk, three or four feet high, and bravely decked with flowers from the middle of the stalk upwards; for on the lower part of the stalk, there is neither branches nor leaf. The flowers are hooded and gaping, being white in colour, and standing in brownish husk, with a long small undivided leaf under each leaf; they seldom seed in our country. Its roots are many, great and thick, blackish without and whitish within, full of a clammy sap; a piece of them if you set it in the garden, and defend it from the first Winter cold will grow and flourish.

They are only nursed in the gardens in England, where they will grow very well. It flowers in June and July.

It is an excellent plant under the dominion of the Moon; I could wish such as are studious would labour to keep it in their gardens. The leaves being boiled and used in clysters, is excellent good to mollify the belly, and make the passage slippery. The decoction drank inwardly, is excellent and good for the bloody-flux: The leaves being bruised, or rather boiled and applied like a poultice are excellent good to unite broken bones and strengthen joints that have been put out. The decoction of either leaves or roots being drank, and the decoction of leaves applied to the place, is excellent good for the king's evil that is broken and runs; for by the influence of the moon, it revives the ends of the veins which are relaxed. There is scarce a better remedy to be applied to such places as are burnt with fire than this is, for it fetches out the fire, and heals it without a scar. This is an excellent remedy for such as are bursten, being either taken inwardly, or applied to the place. In like manner used, it helps the cramp and the gout. It is excellently good in hectic fevers, and restores radical moisture to such as are in consumptions. (Nicholas Culpeper (1616-54) *The Complete Herbal*, 1850.)