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A randomised trial of low dose folic acid to prevent neural tube defects

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Abstract

A randomised trial was initiated in Ireland in 1981 to determine if periconceptional supplementation with either folic acid alone or a multivitamin preparation alone could reduce the recurrence risk of neural tube defects (NTDs) in women with a previously affected pregnancy from 5·0% to 1·0% or less. The trial was concluded before the initial target number of study subjects was reached and without a clear treatment effect being observed. A total of 354 women were randomised to receive one of three treatments: folic acid, multivitamins without folic acid, and folic acid plus multivitamins. At the end of the trial 257 women had had a first trial pregnancy outcome (261 infants/fetuses) where the presence or absence of NTDs was ascertainable. There was one NTD recurrence in the 89 infants/fetuses of women in the multivitamin group and no recurrence in the 172 infants/fetuses of women in the folic acid groups, a non-significant difference. Otherwise eligible women who were pregnant when first contacted constituted a non-randomised control group; there were three recurrences among the 103 infants in this group. The difference in the recurrence rate between the folic acid groups and the non-randomised controls was statistically significant but we have reservations about the validity of this comparison. Although our findings do not provide clear evidence of a protective effect of folic acid supplementation they are consistent with those of the Medical Research Council (MRC) trial which demonstrated the efficacy of folic acid in preventing recurrence of NTDs and they raise the possibility that folic acid may be protective at a much lower dosage than that used in the MRC trial.

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In 1980 Smithells *et al* reported the results of a non-randomised study showing a reduction in the prevalence rate at birth of neural tube defects (NTDs) from 5% in unsupplemented mothers to 0·6% in mothers given periconceptional vitamin supplementation (Pregnavite Forte F, Bencard).[1] Because of the high frequency of these births defects in Ireland[2] and the problems in drawing conclusions[3] from the studies by Smithells *et al*[1 4] and Laurence *et al*,[5] a randomised double blind trial was launched in Dublin in 1981 to determine if periconceptional supplementation with either folic acid alone or a multivitamin preparation alone could reduce the recurrence risk of NTDs in women with a previously affected offspring. Subsequently a randomised trial examining this issue was initiated in 1983 by the British Medical Research Council (MRC) and the results have recently been published.[6]

Methods

The study was conducted in 12 Irish hospitals, four of which were in Dublin, with the Health Research Board as the coordinating centre. The participating clinicians and hospitals are given at the end of the paper.

AIM AND SAMPLE SIZE

The aim of the trial was to determine if periconceptional supplementation with either folic acid alone or a multivitamin preparation alone would reduce the recurrence risk of NTDs in women with a previously affected offspring from 5% to 1% or less. This was similar to the reduction achieved by Smithells *et al*.[1 4]

Various experimental designs were considered including a randomised four arm factorial study with a placebo group receiving no active treatment. Though the final choice of three groups had certain disadvantages, we felt that the possibility of being assigned to a placebo group could, in the light of the publicity at the time (1981) about Pregnavite Forte F and vitamins generally, be a cause of undue anxiety to participants and could adversely affect recruitment. Thus the main analysis of our study was based on two comparisons. The proportion of births of NTDs occurring to women taking multivitamins (two thirds of the sample) was compared with the proportion occurring to women not taking multivitamin tablets (one third of the sample) and the second comparison was similarly between women receiving folic acid and those not receiving folic acid.

On the assumption that either folic acid alone or multivitamins alone reduced the recurrence rate of NTDs from the population level at that time[7] of 5·0% to 1·0%, a total sample of 462 pregnancy outcomes (154 in each group) was required for our trial to have an 80% chance of detecting this at a 5% level of statistical significance.

Ethical approval for the trial was obtained from each participating hospital and recruitment commenced in December 1981. We estimated that our target number of informative (presence or absence of NTD ascertainable) pregnancies might be reached by 1989 but unanticipated declines of 65% in the prevalence

at birth of NTDs and 20% in the number of births in the Dublin maternity hospitals over the study period greatly reduced the number of affected births and when the rate of recruitment fell to an average of 1·3 women per month in late 1987 it was decided to end the trial. Randomisation ceased in January 1988 and 30 April 1990 was set as the closing date for the entry of pregnancy outcomes to data analysis.

ENTRY AND EXCLUSION CRTIERIA

Women who had had a baby with a NTD and who were not pregnant when contacted by the study team but were planning a further pregnancy were eligible for inclusion. A NTD was defined as anencephalus, iniencephalus, encephalocoele, and spina bifida aperta. Women with conditions likely to result in impaired absorption from the gastrointestinal tract were excluded.

RECRUITMENT AND RANDOMISATION

Potential study subjects were identified by means of registers of cases of NTDs in participating hospitals. Eligible women were invited to discuss the trial and were given relevant information. Women who entered the trial gave verbal consent and were randomly allocated to one of three treatment groups: folic acid only (group F), multivitamins excluding folic acid (group M), and folic acid plus multivitamins (group FM). Random allocation was achieved by using consecutively numbered, opaque, sealed envelopes. Block randomisation was employed, 12 subjects per block, stratified by hospital.

TRIAL MEDICATION

The study was designed as a double blind trial. Beecham Pharmaceuticals (UK) initially provided similarly presented white multivitamin tablets without folic acid (Pregnavite Forte) and with folic acid (Pregnavite Forte F—as used by Smithells *et al*[1 4]). Glaxo (Ireland) Ltd manufactured a matched folic acid tablet. Although Beecham Pharmaceuticals (UK) initially agreed to supply the tablets for the full duration of the study their support was withdrawn one year later when 55 mothers had been randomised. Thereafter the commercially available Pregnavite Forte F (purple colour) was used and Antigen Pharmaceuticals produced a white multivitamin tablet without folic acid. It is felt that this partial loss of blinding did not materially affect the study outcome.

Trial participants were instructed to take one tablet three times daily for at least two months before conception and until the date of the third missed period. Compliance was based on tablet counts and blood tests but these results are not reported here. Tablets for women in the folic acid groups provided a daily dose of 0·36 mg of folic acid (0·12 mg/tablet). Tablets in the multivitamin groups provided daily doses of vitamin A 4000 IU, calciferol 400 IU, thiamine hydrochloride 1·5 mg, riboflavine 1·5 mg, pyridoxine hydrochloride 1 mg, nicotinamide 15 mg, ascorbic acid 40 mg, calcium phosphate 480 mg, and ferrous sulphate 252 mg.

Throughout the trial Hoffman La Roche in Switzerland and Glaxo in Ireland carried out regular assessments of the stability of the multivitamin and folic acid tablets, respectively.

STATISTICAL ANALYSIS

Statistical analysis was based on 95% confidence intervals (CI) and two sided hypothesis tests with a 5% level of significance. For continuous variables the Student's t distribution was employed for significance tests. For the analysis of 2×2 tables comparing rates in two groups, an uncorrected χ^2 test was employed in all situations. Use of the uncorrected χ^2 gave rise to some statistically significant effects in situations where the usually accepted method, Fisher's exact test, failed to do so. There is now, however, an increasing body of literature which states that neither Fisher's exact test nor Yates's corrected χ^2 are appropriate tests for tables arising from particular types of study design including clinical trials.[8] It is now suggested that the uncorrected χ^2 should be used for all such 2×2 tables including those with small numbers where expected frequencies fall below five.[9] Group comparisons in 2×2 tables were based on the difference between the component rates or proportions. The CI were test based[10] using the uncorrected χ^2 test. Social class, based on husband's occupation, was coded using the Irish Central Statistics Office's social class code.[11]

Results

During December 1981-January 1988, 354 eligible women were randomised as follows: 115 to the folic acid group (F), 119 to the multivitamin (no folic acid) group (M), and 120 to the folic acid plus multivitamin group (FM). Altogether 81·4% of the women were randomised in the four Dublin hospitals.

At the time of contact by the study office 116 otherwise eligible women were pregnant and thus could not be randomised. Ten of these women entered the trial after this pregnancy and they are included in the analysis as trial participants only. The remaining 106 are included as a non-randomised control group.

Selected baseline data and pregnancy status at the end of the trial are presented for the three randomised groups and the non-randomised control group in table 1. When the trial ended in April 1990, 263 women who remained in the trial had completed a pregnancy, 16 women were not pregnant, and 75 had withdrawn from the study (table 1). After withdrawal, 18 of the 75 women completed a pregnancy before the end of the trial, five becoming pregnant within six months of withdrawing (table 1). Thus 281 randomised women had completed a pregnancy.

Seventy five women withdrew from the trial for the following reasons. Sixty six women said that they were no longer interested in a further pregnancy. The reasons given were: family or marital problems (n=7), fear of recurrence (n=7), advanced maternal age (n=4), and medical problems such as hysterectomy (n=5),

infertility (n=5), and other medical problems (n=12). Twenty six gave no specific reason. Side effects of the study treatments were mentioned by two women. Four women had to be withdrawn from the study because they wanted to take Pregnavite Forte F. There were no significant differences between the randomised groups in reasons for withdrawal. Three women who could not be contacted for compliance visits eventually had to be withdrawn from the trial and were lost to follow up.

The outcomes of the first pregnancy in the trial for randomised women and the non-randomised control pregnancies are shown in table 2. Sixteen of the 25 spontaneous fetal deaths were examined by a pathologist and it was possible to determine the presence or absence of NTDs in two cases (that is, two informative pregnancies); neither case had a NTD. Recurrence rates of NTDs are expressed as proportions of infants/fetuses in informative pregnancies. There was no recurrence of NTDs among the 172 infants/fetuses in the two groups on folic acid compared with a single NTD recurrence (a stillbirth with anencephalus) in the 89 infants/fetuses in the non-folic acid (multivitamin only) group (rate difference 1·1%; 95% CI 0·4 to 2·4%). In the two multivitamin groups the recurrence rate was 1/176 and there was no recurrence in the 85 infants/fetuses in the non-multivitamin (folic acid only) group (rate difference 0·6%; 95% CI −1·0 to 2·2%).

There were three recurrences in the 103 infants/fetuses in the non-randomised controls. The difference in the recurrence rate between the folic acid groups (no recurrence) and these non-randomised controls was significant (rate difference 2·9%; 95% CI 0·4 to 5·4%). Significance was not evident, however, when the non-folic acid group was combined with these non-randomised subjects to give a recurrence of 4/192 (rate difference 2·1%; 95% CI −0·1 to 4·2%). There were two stillbirths (anencephalus and a baby with polycystic kidneys and cleft lip) in the multivitamin group (2/96=2·1% of all infants/fetuses) and none in the folic acid groups (0/189) (rate difference 2·1%; 95% CI 0·03 to 4·2%). Other fetal and neonatal outcomes are presented in the appendix.

Discussion

The purpose of this trial was to examine the

Table 1 Baseline characteristics and pregnancy status at end of trial for trial and non-randomised control groups

	Randomisation group				*Non-randomised controls (n=106)*
	Folic acid (F, n=115)	*Multivitamins (M, n=119)*	*Folic acid plus multivitamins (FM, n=120)*	*All randomised subjects (n=354)*	
Maternal age (years) mean (SD)	31·9 (5·0)	30·6 (4·3)	31·4 (4·5)	31·3 (4·6)	31·3 (5·0)
No (%) married	114 (99·1)	117 (98·3)	118 (98·3)	349 (98·6)	105 (99·1)
No (%) in social classes 5 and 6	22 (19·1)	29 (24·4)	26 (21·6)	77 (21·8)	38 (35·9)*
Previous obstetric history					
Mean (SD) No of:					
Births of NTDs	1·07 (0·26)	1·04 (0·24)	1·07 (0·28)	1·06 (0·26)	1·04 (0·19)
Spontaneous abortions	0·63 (1·00)	0·63 (1·06)	0·53 (0·93)	0·59 (1·00)	0·58 (1·14)
Total pregnancies	2·98 (1·78)	2·95 (1·77)	3·03 (1·72)	2·99 (1·75)	3·91 (2·14)*
No (%) with spontaneous abortion in immediately preceding pregnancy	20 (17·4)	14 (11·8)	14 (11·7)	48 (13·5)	9 (8·5)
Pregnancy status at close of trial					
No (%) pregnant	93 (80·9)	95 (79·8)	93 (77·5)	281 (79·4)	106
Non-withdrawal	85	93	85	263	
Withdrawal	8	2	8	18	
No (%) not pregnant	22 (19·1)	23 (19·3)	25 (20·8)	70 (19·8)	
Non-withdrawal	4	4	8	16	
Withdrawal	18	19	17	54	
Pregnancy status not known in withdrawals	—	1 (0·9)	2 (1·7)	3 (0·8)	

*Randomised subjects *v* non-randomised controls: p<0·01.

Table 2 Pregnancy outcome in trial and non-randomised control groups

	Randomisation group				*Non-randomised controls (n=106)*
	Folic acid (F, n=115)	*Multivitamins (M, n=19)*	*Folic acid plus multivitamins (FM, n=120)*	*All randomised subjects (n=354)*	
Informative outcome (total)	85 [1]	89 [1]	87 [2]	261 [4]	103 [3]
No (%) of:					
Livebirths	85 (90·4) [1]	87 (90·6) [1]	85 (89·5) [2]	257 (90·2) [4]	99 (90·8) [3]
Stillbirths	—	2 (2·1)	—	2 (0·7)	4 (3·7)*
Spontaneous abortions	—	—	2 (2·1)	2 (0·7)	—
Non-informative outcome (total)	9	7	8	24	6
No (%) of:					
Spontaneous abortions	9 (9·6)	7 (7·3)	7 (7·4)	23 (8·1)	6 (5·5)
Ectopic	—	—	1 (1·0)	1 (0·3)	—
Total No (%) of infants/fetuses	94 (100·0) [1]	96 (100·0) [1]	95 (100·0) [2]	285 (100·0) [4]	109 (100·0) [3]
Total No of pregnancies	93	95	93	281	106
No of women not pregnant	22	23	25	70	—
Pregnancy status unknown	—	1	2	3	—

Unless otherwise stated, the data relate to infants/fetuses resulting from the first pregnancy on the trial. Figures in lower parentheses [ ] indicate numbers of twin pairs included.
No statistically significant differences between randomised groups.
*Randomised subjects *v* non-randomised controls: p<0·05.

results of Smithells *et al*[1] using a randomised design. Due to a marked fall over the study period in the number of eligible participants, it became necessary to end the trial prematurely without the initial target number of participants being reached and without a treatment effect being clearly established.

There was one NTD recurrence in this trial which occurred in the non-folic acid group. There were no significant differences in recurrence between the randomised groups. With only one recurrence it is obviously difficult to draw any major conclusions. The difference in the recurrence rate between the folic acid groups and the non-randomised controls was statistically significant (p=0·02) but when the multivitamin only group (who did not receive folic acid supplementation) was combined with the non-randomised controls (who probably did not receive folic acid supplementation), the difference just failed to reach statistical significance (p=0·06). This analysis is contingent on accepting the appropriateness of the statistical test used (see methods). Furthermore, as this is a comparison between a randomised group and a non-randomised control group, we feel that this finding should be interpreted with great caution. Despite our reservations these results are presented in view of the important question of high versus low dose folic acid in preventing NTDs.[12]

The very low recurrence rate in the randomised women (0·4%) may have been due to self selection into the trial by women with a low recurrence risk. A comparison between the randomised women and the non-randomised controls for the risk factors for recurrence showed no consistent differences between the groups, although the control women had more previous births and were more likely to be in the lower social classes. Other confounding factors not investigated, for example, diet, may have contributed to the difference in recurrence rates. Baseline characteristics were similar for the three randomised groups, however.

Our findings are consistent with those of the MRC trial which demonstrated the efficacy of folic acid in preventing NTD recurrence.[6] The absence of any NTD in the 169 women allocated to folic acid suggests that folic acid may also have been protective in our study. Should this be so, it raises the important consideration that folic acid may be protective at a daily dosage of 0·36 mg as used by us as well as at the much higher dosage of 4 mg used in the MRC trial. In the non-randomised studies conducted by Smithells *et al*,[1 4] women supplemented with Pregnavite Forte F, which contained a daily dose of 0·36 mg of folic acid, had a consistently low recurrence rate of 1·0% or less. However, the dangers of comparisons with non-randomised studies are well documented. We emphasise that our findings, taken on their own, do not provide clear evidence of a protective effect of either folic acid or other vitamins.

Based on the criteria for registering congenital malformations by the population based Dublin Eurocat Register of congenital malformations and excluding NTDs, 12 (4·6%) of the babies in the randomised groups had one or more congenital malformations compared with 2·8% of babies in the Dublin Eurocat register during 1982–87 (p=0·08).[13] A possible explanation for the higher rate in the trial is that ascertainment may have been more complete in these babies. It was interesting to note that women in the non-folic acid group had a higher stillbirth rate which was accounted for by two babies with congenital malformations (including the NTD recurrence).

The MRC trial provides evidence that periconceptional supplementation with folic acid reduces the recurrence risk of NTDs and recommends a daily dose of 4 mg of this substance.[6] We propose that this advice should be offered to Irish women, although we have some reservations at the moment about the need for and safety of this high dose of folic acid.[14] Further research on this and other aspects is in progress.

Appendix

FETAL AND NEONATAL OUTCOMES

Among the randomised women there were 13 infants/fetuses reported as having a congenital malformation excluding the NTD recurrence (a stillbirth with anencephalus) but including the other stillbirth with polycystic kidneys and cleft lip. There were seven major malformations: one in group F (bilateral corneal ectasia with agenesis of the corpus callosum), four in M (polycystic kidneys with cleft lip, congenital mitral insufficiency, polydactyly, and pyloric stenosis), and two in FM (urethral obstruction and cystic fibrosis). Six minor malformations were reported: four cases of congenital dislocation of the hip (one in group F and three in group FM), talipes (group F), and scaphocephaly (group M). Three babies had anomalies of interest in that they might possibly be related to NTDs. One baby in group F had a postanal dimple; a baby in group M had a pilonidal sinus at the base of the lumbar spine and a radiograph showed slight widening of the lumbar spine; a baby in group FM had a sacral naevus without underlying bony defect seen on radiography. Three major congenital malformations were reported in the control group: hydrocephalus (without spina bifida), oesophageal atresia, and transposition of the great vessels. During the neonatal period one baby in group M developed *Escherichia coli* septicaemia and another in group FM had necrotising enterocolitis.

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The coordination centre was the Health Research Board, Dublin; Dr P N Kirke, Ms J Murtagh, Ms P Lawler, Ms B Fitzpatrick, and Ms H Burke.

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If children got more calcium

If children and adolescents have a relatively high intake of calcium then peak bone mass (PBM) achieved in early adulthood is likely to be greater.

If PBM is higher, then bone mass in later life is also likely to be higher and fractures in middle and old age less common.

If hip fractures occur in people with osteoporosis there may be an associated mortality of up to 20%.

If children develop a dietary habit it may persist when they are adults.

If people take more exercise, that too leads to them having a greater bone mass.

If the recommended dietary intake of calcium in childhood is increased will it be wholly beneficial.

If children take more calcium they are likely to do so by taking more milk. Would that be entirely a good thing?

If one of a pair of identical twins is given capsules of calcium citrate malate daily for three years and the other twin is given placebo then, provided they remain prepubertal at the end of the three years, the one given extra calcium will achieve a greater bone density (C Conrad Johnston Jr and colleagues, *New England Journal of Medicine* 1992;**327**:82–7.

If I were a parent of twins I think I'd want to protect them from researchers.

If calcium is present in the human body with the same relative abundance as in the earth's crust and the ratios of the various isotopes of calcium in all things terrestrial were determined 4·5 billion years ago (Velimir Matkovic, leading article, *New England Journal of Medicine* 1992;**327**:119–20) how could anybody top that as a piece of information?

ARCHIVIST

If Rudyard Kipling is looking down from above I'm sure he'll understand.