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The Role of Folic Acid in Oral Clefting

T. HARTRIDGE, B.D.S.
Maxillofacial Unit, Royal Devon & Exeter Hospital, Barrack Road, Exeter, UK

H. M. ILLING, M.SC., M.ORTH., F.D.S.R.C.S.
Maxillofacial Unit, Queen Alexandra Hospital, Cosham, Portsmouth, UK

J. R. SANDY*, PH.D., M.ORTH., F.D.S.R.C.S., F.D.S.R.C.S., F.F.D.R.C.S.I.
Division of Child Dental Health, University of Bristol Dental School, Lower Maudlin Street, Bristol BS1 2LY, UK

Abstract: *The objective of this study is to describe the role of periconceptional folic acid supplementation and assess it's potential in the prevention of foetal abnormalities, and consists of a review of the literature undertaken using an electronic and hand search. This includes research trials and methodology associated with folic acid supplementation.*

It is recommended that all women planning to conceive should supplement their diet with folic acid in order to prevent abnormalities in neural tube development, particularly if there is a history of a previously affected pregnancy. There is increasing evidence that folic acid supplementation may, in addition, reduce the incidence of oral facial clefting. Further research with multi-disciplinary approaches in biochemistry, genetics, gene/environment interactions, and embryology are indicated.

Refereed Paper

Introduction

It had been suspected for many years that a mother's periconceptional diet may have a role in the causation of birth defects. In 1991, a multi-centre recurrence study by the MRC vitamin study research group (MRC Vitamin Study Research Group, 1991) produced good evidence to recommend folic acid supplements to prevent neural tube defects in mothers who previously had an affected pregnancy. Other studies (case control and controlled trials) have shown this protective effect for mothers with no history of affected pregnancies (Bower and Stanley, 1996).

Not surprisingly, since facial mesenchyme is derived from neural crest, it was postulated that periconceptional folic acid supplementation may reduce the occurrence of offspring with orofacial clefts. Oral clefts (cleft lip, cleft palate, and cleft lip and palate) are one of the most common congenital malformations with an approximate prevalence of 1·5 per 1000 live births (Owens *et al*., 1985). It is thought (Tolarova, 1987; Schuber *et al*., 1990) that a multi-factorial model can explain the aetiology of oral clefts, liability depending on genetic (endogenous) and non-genetic (exogenous) factors. This review primarily examines the current clinical studies investigating the effects of folic acid on the incidence of oral clefts and other birth defects. Evidence and further investigation from other sources, including genetics, environment/gene interaction, biochemistry, and embryology, are required to fully understand the role played by folic acid in oral clefts.

If a protective effect of folic acid in oral clefting were proven, it would have the following implications:

1. A reduction in the incidence of oral clefts and, therefore, positive social, physical, psychological, and financial consequences.
2. Further promotion of folic acid supplementation to women planning a pregnancy.
3. Strengthen the case for food fortification with folic acid (McNulty, 1995). This is important since many pregnancies are unplanned.
4. Allow women who have a high risk of producing offspring with oral clefts to be targeted with folic acid supplementation (mothers who already have a cleft-affected child or those on medications which may induce oral clefts). Anti-convulsants including valproate, carbamazepine, and phenytoin (Saxen, 1975; Hill *et al*., 1988) and methotrexate (Milunsky *et al*., 1968) have been observed to induce oral clefts in humans.
5. Promote research into mechanisms of cleft formation to further preventative strategies or treatment techniques.

Folic Acid

Naturally occurring folates are found widely in foodstuffs, especially in liver, yeast extract, legumes, and fresh green-leafed vegetables. Folic acid (pteroylmonoglutamic acid) is a commercially available compound used for supplementation or food fortification (Christensen and Rosenblatt, 1995; McNulty, 1995; Zittoun, 1995). It does not occur naturally in living tissues, but is readily converted *in vivo* into the biologically active folates. The supplementation of folic acid currently recommended to protect against neural tube defects is 0·4 mg per day, twice the current average daily intake for women of 0·2 mg (McNulty, 1995), there being a wide variation in dietary intake. Other factors including age, sex, race, drugs, folate absorption, transport, and utilization, can explain the wide variation of folate status seen. It is unrealistic to expect many of the population to achieve this recommended folic acid intake through diet alone. Therefore, supplementation or fortification of staple foodstuffs is the only realistic option of increasing daily intake of folic acid. Little is known about

*To whom correspondence should be addressed.
E-mail: Jonathan.Sandy@bris.ac.uk

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the diet or folate status of mothers of children with orofacial clefts.

In combination with specific enzymes, folates act as coenzymes in the transfer (acceptor or donor) of one-carbon units in many biochemical reactions involving amino acid metabolism. Folates are essential in the synthesis of purines and pyrimidines, which are components of DNA and RNA required in the regulation of gene expression and cell differentiation. Rapidly proliferating tissues have the greatest requirement for DNA synthesis and, therefore, a deficiency of folates will be first seen in the haematopoietic system (resulting in anaemia), epithelial cell surfaces, and gonads. Folate deficiency also results in elevation of homocysteine due to failure of its remethylation by folate dependant enzymes. A rise in homocysteine levels has been linked to an increased risk of neural tube defects (Ubbink, 1995).

Folic Acid and Neural Tube Defects

Both neural tube defects and oral clefts are thought to have a multifactorial inheritance, and occur more frequently together than can be expected by chance alone (Khoury *et al*., 1989a). Neural crest cells have a role in both neural tube closure and in the development of the oral structures, which again suggests an association between the two malformations. It is generally agreed (Larsen, 1993) that the neural walls fuse on day 21–22 after conception, the cranial neural pore closes at day 24, and the caudal neural pore at day 26. Defects of neural tube closure result in various malformations or the spontaneous abortion of the foetus. Failure of closure of the cranial neural pore results in malformations, such as anencephaly; failure of caudal pore closure results in spina bifida. Neural tube closure is complete very early in pregnancy when most mothers have barely realized they are pregnant.

The incidence of neural tube defects ranges from 0·6 to 3·7 cases per 1000 live births, and varies between socioeconomic and ethnic groups. Mothers with one or more neural tube defect pregnancies have a 10-fold increase of a subsequent recurrence (MRC Vitamin Study Research Group, 1991). Medication may play a role in the causation of neural tube defects. Certain anti-folate drugs have been shown to be teratogenic and the use of valproate drugs during pregnancy, for the treatment of epilepsy, has been associated with a 5- to 20-fold increase in the incidence of neural tube defects (Baile and Lewenthal, 1984; Dansky *et al*., 1992). Animal models exist where there is a genetic susceptibility to neural tube malformations. If these animals are given anti-folate drugs or a folate deficient diet an increased incidence of neural tube defects is observed (Evans *et al*., 1951; Depaola and Mandella, 1981).

Two studies (Smithells *et al*., 1980; Laurence *et al*., 1981) have suggested that folic acid or other vitamin supplementation may reduce the risk of neural tube defect affected pregnancies in mothers already having one affected offspring. Unfortunately, these studies had low statistical power with which to detect treatment differences. In 1991, the MRC vitamin study research group undertook a randomized double-blind multi-centre trial to assess the effects of folic acid supplementation to mothers already having a child with a neural tube defect (MRC Vitamin Study Research Group, 1991). After 1195 known pregnancy outcomes were recorded (2000 were planned), there was a 72 per cent reduction in neural tube defects in the mothers treated with folic acid. With this evidence, it was considered unethical to withhold the treatment from the control sample and the study was therefore terminated.

This research helped to provide the evidence for the current recommendation that women with a previously affected pregnancy should take 4 mg folic acid daily (5 mg folic acid is the only suitable preparation currently available in the UK) and all other women planning to conceive 0·4 mg folic acid from preconception to the twelfth week of pregnancy.

Folic Acid and Oral Clefts

Various types of studies can be examined to evaluate the role of folic acid in oral clefting. The most carefully controlled studies involve animal models, where an increased risk of oral clefting in animals given folate deficient diets or anti-folate drugs has been observed. There is also a protective effect with periconceptional folic acid supplementation (Evans *et al*., 1951; Jordan *et al*., 1977; Depola and Mandella, 1981; Elwood and Colquhoun, 1997).

In humans, drugs which interfere with folate metabolism, for example, phenytoin, are known to have teratogenic effects. These include oral clefts, growth retardation, limb defects, and other craniofacial deformities. Folate antagonists have also been used as agents to induce abortions in humans (Thiersch, 1952). In a prospective study on epileptic women taking anti-folate drugs (Danskey *et al*., 1992), blood folate levels reduced with increasing levels of anti-epileptic drug. Low blood folate levels were associated with spontaneous abortion and developmental abnormalities of the foetus.

A human trial on mothers who at the time of pregnancy were receiving anti-convulsive drugs demonstrated a protective effect of folic acid on oral clefting (Baile and Lewenthal, 1984). None of the 33 offspring of the mothers receiving folic acid supplementation showed developmental anomalies. This compared to 15 per cent of the 66 offspring in the control group, where the mothers had not received folic acid supplementation. The deformities in the newborn included oral clefting.

There appears to be an association between maternal smoking and clefting. Khoury *et al*. (1989b) found that mothers who smoked were 1·6–2 times more likely to have offspring with isolated oral clefts. Smokers are also known to have significantly lower folate status than non-smokers (McNulty, 1995), but it is not known if the decreased folate levels in smokers is due to decreased folate intake or increased folate requirement. Clearly, there is potential to rectify folate levels in mothers who persist in smoking during pregnancy.

Human studies involving folic acid supplementation may be divided into retrospective case control studies assessing a mothers exposure to folic acid during a pregnancy or prospective trials involving vitamin supplementation to mothers planning a child. It is not appropriate to combine the results of the case control studies with the supplementation trials due to their differing methodologies. The results of the two types of study have therefore been separated.

Case Control Studies

The results of the case control studies are summarized in Table 1.

Out of the seven case-control studies, only two found a significant protective effect for folic acid in oral clefting. Czeizel *et al.* (1996) found the protective effect of folic acid to be significant for all types of oral cleft, whereas Shaw *et al.* (1995) found this only applied to the isolated cleft lip or palate group. The results of case-control studies fail collectively to prove a protective effect for folic acid. Due to the low power of some of the studies and the bias that is inevitably introduced in their methodology, there should be caution in attaching significance to the conclusions.

Prospective Supplementation Trials

The results of the prospective supplementation trials are summarized in Table 2.

All of these trials have demonstrated a protective effect for folic acid on the incidence of oral clefting, the statistical significance of a protective effect has not been proved by all the studies. There are five complete samples, the statistical significance of this protective effect was not calculated in two of the studies (Conway, 1958; Briggs, 1976). The difference between the treatment groups was not statistically significant in the studies of Fraser and Warburton, (1964), and the trials of Czeizel where first time mothers were supplemented (Czeizel and Dudas, 1992; Czeizel, 1993,1998; Czeizel and Hirschberg, 1997). The protective effect of folic acid for oral clefts was shown to be statistically significant in the sample reported by Tolarova (Tolarova, 1982, 1987; Tolarova and Harris, 1995). Efficacy for prevention of cleft recurrence was shown to be greatest for subgroups with unilateral, rather than with bilateral, and male, rather than female probands. It should be noted that Tolarova used an extremely high dose of folic acid in his trials (10 mg), when compared with doses used in other supplementation studies (0·5–5 mg). It has been suggested (Schwartz *et al*., 1950) that large daily doses of folic acid (5 mg/day) can mask the early diagnosis of pernicious anaemia (vitamin B_{12} deficiency) by reducing the asso-

TABLE 1 *Summary of case-control studies (retrospective)*

Study	Sample		Method	Results
	Case	Control		
Bower & Stanley (1992)	59 mothers of infants with midline birth defects (13 oral clefts)	115 mothers of infants without malformations	Interview and questionnaire assessing periconceptional diet and supplemented folic acid	No association between midline defects and either diet or folic acid supplementation
Czeizel *et al.* (1996)	17,300 mothers of infants with congenital abnormalities (1375 oral clefts)	30,663 mothers of infants without malformations	Questionnaire and medical record review assessing folic acid supplementation	Significant protection after folic acid for all oral clefts, cardiovascular and neural tube defects
Fraser & Warburton (1964)	146 mothers of infants with oral clefts (CL/P 187, CP 59)	90 mothers of infants with genetically-determined diseases	Interview assessing periconceptional vitamin supplementation	No significant differences in vitamin supplementation between groups
Hayes *et al.* (1996)	303 mothers of infants with oral clefts (CL/P 195, CP 108)	1167 mothers of liveborn or stillborn infants with a congenital abnormality (oral clefts, neural tube and other midline defects excluded)	Interview and questionnaire assessing periconceptional diet and supplemented folic acid	No significant protective effect of folic acid for all oral clefts
Hill *et al.* (1988)	676 mothers of infants ith oral clefts (CL/P 458, CP 218)	676 mothers of infants without malformations	Medical record review of preconceptional drug history and vitamins prescribed	No significant protective effect of folic acid for oral clefts
Peer *et al.* (1958)	400 mothers of infants with oral clefts	None	Pregnancy questionnaires sent to 1000 mothers of infants with oral clefts	Of 306 cases with negative family history, vitamins not taken in 165 cases, irregular usage in 110 cases
Saxen (1975)	599 mothers of infants with oral clefts	599 mothers of infants without malformations	Interview assessing post conception drug consumption and vitamin supplementation	No significant protective effect of vitamin or iron supplementation for oral clefts
Shaw *et al.* (1995)	731 mothers of infants with oral clefts	734 mothers of infants without malformations	Interview assessing diet, periconceptional vitamin and folic acid supplementation	Reduced risk for all oral clefts with folic acid supplementation. Only significant reduction (50%) in isolated CL/P group

TABLE 2 *Summary of supplementation trials (prospective)*

Study	Sample		Supplement		Results-recurrence/occurrence (all clefts)	
	Supplemented	Control	Supplemented	Control	Supplemented	Control
Conway (1958)	39 mothers 59 births	48 mothers 78 births	MV/ 0·5 mg FA	None	0% (0 cases)	5·1% (4 cases)
Fraser & Warburton (1964)	156 mothers 156 births	383 mothers 383 births	Vitamins, type not stated	None	1·9% (3 cases)	5·7% (22 cases)
Peer & Gordon (1964)	176 mothers 176 next born 206 all births	418 mothers 418 next born	MV/ 5 mg FA	None	2·3% (4 cases) next born 1·9% (4 cases) all births	4·8% (20 cases)
Briggs (1976) (includes Peer, 1964 sample)	228 mothers 228 next born 348 all births	417 mothers 417 next born	MV/ 5 mg FA	None	3·1% (7 cases) next born 3·2% (11 cases) all births	4·8% (20 cases)
Tolarova (1982)	80 mothers* 84 births	202 mothers 206 births	MV/ 10 mg FA	None	1·2% (1 case)	7·3% (15 cases)
Tolarova (1987) (includes Tolarova, 1982 sample)	184 births*	1901 births	MV/ 10 mg FA	None	1·6% (3 cases)	4·0% (77 cases)
Tolarova & Harris (1995) (includes Tolarova, 1982 and Tolarova, 1987 sample)	214 births*	1901 births	MV/ 10 mg FA	None	1·4% (3 cases)	4·0% (77 cases)
Czeizel & Dudas (1992) Czeizel (1993) Czeizel & Hirschberg (1997) Czeizel (1998)	2471 births†	2391 births	MV/ 0·8 mg FA	TE	0·16% (4 cases)	0·21% (5 cases)

† sample from mothers already having a cleft affected child or mother/father having a cleft lip and/or palate
* sample from first time mothers
All other study samples are from mothers already having a cleft affected child
MV multivitamin, FA folic acid, TE trace element supplement

ciated macrocytic anaemia, but allow neurological damage to continue. Tolarova and Harris (1995) monitored the blood count before and during folic acid supplementation, which he suggested would alert the clinician to the potential adverse effects of the high dose folic acid used.

There are a number of possible explanations why these case control studies and supplementation trials have failed to conclusively prove a protective effect for folic acid in oral clefting:

The studies have lacked statistical power to prove a protective effect. This may be due to:

1. Small sample numbers.
2. Low incidence of oral clefting in the population studied.
3. Folic acid provides only a partial protective effect.

Study bias:

1. Incorrect classification of pregnancy outcome. Some studies included observed deformities of still born or aborted offspring, others failed to include this outcome.
2. Lack of randomization/blindness in some prospective trials.
3. Patient compliance in taking supplements or in case control studies, the ability to assess accurately the mothers periconceptional exposure to folic acid.
4. Unmeasured confounding, studies failing to assess mothers exposure to possible teratogens, such as smoking, drugs, alcohol, and stress.
5. Differing protective effect of folic acid within a population due to genetic and other environmental interactions.
6. Possible affects of folic acid on foetal survival or spontaneous abortion, thereby affecting the incidence of oral clefts in a liveborn sample.

Statistical methods used in the analysis of results. The detection of small differences between two small samples often tests statisticians and their methods.

Future Research

A study with sufficient power would require a large multicentre randomized double blind trial on mothers at higher risk of oral clefts, similar to the MRC study into folic acid and neural tube defects. Withholding folic acid from mothers to form a control group would, however, be unethical.

A trial could supplement all prospective mothers with folic acid of differing daily dosage to investigate dose–response for the affects of folic acid. Large cleft susceptible populations would, however, be required.

Case control studies which have been used to investigate neural tube defects could be repeated for oral clefts. This would include investigation of maternal and foetal folate status, and ability to absorb and metabolize folate in

mothers and their cleft affected offspring. Lower red cell and serum folate levels have been found in mothers of children with neural tube defects compared to control mothers (Bunduki *et al.*, 1995; Wald *et al.*, 1996).

Conclusions

Current recommendations to prevent neural tube defects, advise all women planning to conceive a child to supplement their diet with 0·4 mg folic acid from preconception to the twelfth week of pregnancy. Women with a previously affected pregnancy should take 4 mg folic acid daily. The protective effect for periconceptional folic acid on oral clefting has not been proved conclusively. However, studies have found significant protective effects and the above guidelines should be adhered to. Further research into the role of folic acid in orofacial clefting is indicated.

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