Mung Bean Flour Increases Blood Folate Levels in Pregnant Mice Model of Cleft Lip and Palate

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Abstract

To analyse mung bean flour (MBF) administration to increase blood folate levels in pregnant mice models of cleft lip with or without cleft palate (CL/P).

An experimental study was conducted using Balb/C mice. Pregnant mice were randomly separated into five groups. The mice that received distilled water were used as negative control (C-), while mice in the other four groups (one positive control group (C+) and three treatment groups) were given with phenytoin of 60 mg/kg at gestational day (GD) 10 intraperitoneally and the three treatment groups were added with MBF 0.25 g, 0.5 g and 1 g/day, respectively. The MBF was given from GD 0 until the sampling data were collected at GD 17.

Blood folate levels in T1 (0.25 g/d MBF), T2 (0.5 g/d MBF) and T3 (1 g/d MBF) groups were higher than those in C- and C+ groups. Anova test results on the comparison of the effect of MBF on blood folate levels in mice showed a significant difference with $p = 0.037$ ($p < 0.05$). There was a significant difference the effect of MBF between C- and T1 (0.25 g/d MBF) groups, between C- and T3 (1 g/d MBF) groups, between C + and T1 (0.25 g/d MBF) groups, and between C + and T3 (1 g/d MBF) groups, but there was no significant difference between the C- and C + groups, and between T1 (0.25 g/d MBF), T2 (0.5 g/d MBF) and T3 (1 g/d MBF).

Mung bean flour administration could increase blood folate levels in mother mice models of CL/P.

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Introduction

The non-syndromic (NS) cleft lip with or without cleft palate (CL/P) is one of the most common congenital defects. The prevalence of CL/P varies from 3.4 to 22.9 per 10,000 births worldwide, depending on factors such as ethnicity, geographic location and socioeconomic status. East Asian and Native American populations have the highest incidence of CL/P, while African populations have the lowest. Asians have the highest risk in 14 : 10000 births. Caucasians have in 10 : 10 000 births, followed by African American births of 4 : 10000[1,2].

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One of the most important factors related to the incidence of CL/P is diet, because the diet of pregnant women affects fetal growth. Leite et al., 2002 found that there was a 25-50% reduction in the risk of CL/P in mothers who had taken multivitamins containing folic acid during a period of one to two months after conception [3]. Lack of folate intake during pregnancy increases the risk of having a child with CL/P[4]. This is related to the role of folate as a one-carbon donor in purine and pyrimidine biosynthesis and homocysteine remethylation, resulting in methyl groups for DNA, protein, and lipid methylation [5,6,7].

The incidence of NS CL/P is even more common in people who are less accessible to the health system due to geographic difficulties[8]. Nutrition intake of folate should reach all levels of society in the prevention of NS CL/P[2]. One of the natural source of folate is mung bean seeds (*Vigna radiata L.*) [9]. The content of vitamin B9 (folic acid) in a serving of 1 cup of mung bean seeds (*Vigna radiata* L.) boiled without salt fulfils 80% of the need for folic acid in one day[10].

In view of this benefit of mung bean as the natural source of folate and the importance to reduce the incidence of cleft lip and palate, the provision of mung bean for pregnant mothers and its effect on cleft incidence should be investigated. The aim of this study was to analyse mung bean flour administration to increase blood folate levels in the mother of mice models of CL/P.

Materials and methods

Mung bean flour preparation

The mung bean flour (MBF) was prepared using the following methods: The beans were sorted, washed, and dried, and the particle size of the bean was reduced using disk mill and sieved using an 80 mesh sieve. Per gram of mung bean seeds contains 6.87 µg of folate. The folate requirement for pregnant mice was 1.56 µg/day = 0.227 g/day = ± 0.25 g/day. The mung bean flour was dissolved in normal saline solution with Carboxy Methyl Cellulose (CMC) solvent in concentrations of 0.25 g/mL, 0.5 g/mL, and 1 g/mL for feeding in the following in vivo experiment in doses of 0.25 g/day, 0.5 g/day, and 1 g/day.

Mice model of CL/P

This institutionally approved study used Balb/C mice. Ovulation was induced in female mice by injecting 5 IU of pregnant mare serum gonadotropin (PMSG) followed by 5 IU of human chorionic gonadotropin (hCG) 48 hours later. Immediately after hCG injection, the female were placed with the male at a 1 to 1 ratio for 24 hours. Pregnant mice were randomly separated into five groups, with 10 mice in each group (n = 10). Mice given with distilled water were used as negative control (C-). Mice in the other four groups (one positive control group (C+) and three treatment groups) were given with phenytoin of 60 mg/kg at gestational day (GD) 10 intraperitoneally and in three treatment groups the MBF was added in doses of 0.25 g, 0.5 g and 1 g/day respectively. Mung bean flour was given from GD 0 until sampling data were collected at GD17. Using a 1 ml syringe, ± 1 ml of blood from pregnant mice was taken from the left ventricle. The blood was drawn from the main heart, put into a vacuum tube, with ethylenediamine tetraacetic acid (EDTA), labeled and prepared to measure the folate levels. The folate levels were measured according to the instructions attached to the mouse folic acid ELISA Kit (catalog number MBS723274).

Results

Observation group	Average ± SD	*p-value Anova*
C-	0.37±0.10[a]	P=0.037 (p<0.05)
C+	0.38±0.15[a]	
T1 (0,25 gr/d MBF)	0.51±0.11[b]	
T2 (0,5 gr/ d MBF)	0.47±0.10[ab]	
T3 (1 gr/d MBF)	0.50±0.07[b]	

Table 1. The results of the comparative test on the effect of mung bean flour on blood folate levels.

Anova test results on the comparison of the effect of mung bean flour on blood folate levels in mice showed a significant difference with p = 0.037 (p <0.05). Different letter notations indicate significant differences.

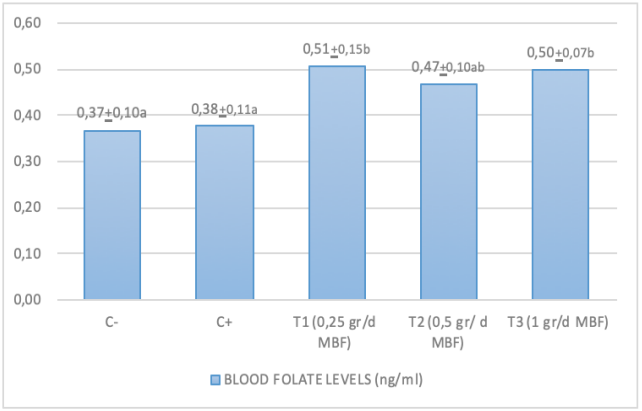

Figure 1. Diagram of mean blood folate levels.

The mean blood folate levels as shown in Figure 1 show that the blood folate levels in the T1 (0.25 g/d MBF), T2 (0.5 g/d MBF) and T3 (1 g/d MBF) groups were higher than those in C- and C+ groups. Based on the LSD test, different letter notations showed significant differences between groups. So it appears that no significant differences were found between the C- and C + groups, and between T1 (0.25 g/d MBF), T2 (0.5 g/d MBF) and T3 (1 g/d MBF). There was a significant difference between C- and T1 (0.25 g/d MBF) groups, between C- and T3 (1 g/d MBF) groups, between C + and T1 (0.25 g/d MBF) groups, and between C + and T3 (1 g/d MBF) groups.

Discussion

Anova test results comparing the effect of MBF on blood folate levels in mice showed a significant difference with p = 0.037 (p <0.05). The mean blood folate levels in the CL/P model mice showed an increase in folate levels in all treatment groups, when compared to the control group, C- and C+ , although C- and C+ were not significantly different from T2 (0.5 g/day MBF). This proves that the administration of MBF to the treatment group increases blood folate levels.

A critical review by Ganesan and Xu (2017) states that folate levels in mung bean seeds are 625 µg per 100 grams [10,11]. The high levels of folate and protein content in mung bean seeds make it a potential food choice that has high nutritional levels[12]. Research by Varma et al., 2018 regarding the biochemical composition and protein profile of mung bean cultivar storage states that mung beans are rich in dietary fiber, carbohydrates, energy, vitamins and minerals, ie. iron, magnesium, phosphorus, potassium, copper and folate; while riboflavin and niacin are found in small amount[9]. Adequacy of blood folate levels is believed to reduce the risk of fetal defects, because women with folate deficiency may be more susceptible to epigenetic effects from environmental exposure [13].

Another supporting study is a study by Zhang et al., 2019 which proved that perinatal folic acid supplementation can reduce the risk of various birth defects, including CL/P. Although its mechanism of action has not been fully elucidated, folic acid may be a scavenger that directly regulates maternal ROS levels during pregnancy. Mitochondria are known to be the targets of folic acid in the stem cells of primary teeth of normal children. The study found that the anti-oxidant effect of folic acid also involves mitochondrial activation, including ATP production in CBL. In addition, the expression levels of not only SOD1 but also SOD2 did increase in folic acid supplemented CBLs, suggesting that folic acid has an anti-oxidant effect via an increase in the associated protein. Although gene expression can be regulated by many factors, folic acid contributes to epigenetic modifications, including DNA and histone methylation via one-carbon metabolism. Thus, epigenetic regulation of SOD transcription in the downstream one-carbon metabolic pathway initiated by folic acid explains the positive effect of perinatal folic acid supplementation on anti-oxidant activity in neural crest cells, thereby reducing the risk of CL/P [14,15].

In this study, since we found no significant difference between the C- and C+ groups, this proved that phenytoin administration had no effect on blood folate levels. This was not in accordance with research by Linnebank et al., 2011 which proved that antiepileptic drugs interacted with folate and serum vitamin B12 levels. The study found evidence that treatment with carbamazepine, gabapentin, oxcarbazepine, phenytoin, primidone, or valproate was associated with lower mean serum folate levels compared with all groups of patients, untreated patients, or controls. Levels of folate or vitamin B12 below the reference range were associated with higher mean corpuscular volume (MCV) and higher plasma homocysteine levels [16].

Conclusions

Mung bean flour administration could increase blood folate levels in pregnant mice models of CL/P.

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Author Contributions

RRA directed the study; conceived and designed the study; collected the data; wrote the paper. DSP encouraged and supervised the findings of this work. RH encouraged and verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

Declaration of Interest

The authors report no conflict of interest.

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