The Effect of DFMO on Polyamine Levels in Colon Polyp Tissue in a Randomized Clinical Trial

Group 10

Summary

Difluoro Methyl Ornithine (DFMO) is a pharmacological agent undergoing clinical trials for the reduction of polyamine levels in colon polyp tissue. In order to assess the effect of DFMO on polyamine levels in colon polyp tissue, a 15-month randomized placebo controlled clinical trial, involving 12 months of treatment with DFMO and 3 months of follow-up after cessation of treatment, was conducted among 114 individuals with a history of colon polyps. Participants were randomized into 4 groups (placebo, 0.075, 0.20 and 0.40 g/sq m/day of DFMO). Biopsies of colon tissue were obtained at 0, 6, 12, and 15 months and levels of putrescine, spermidine, and spermine (µmol/mg protein) were measured at these times. Using two-sample t-test, the geometric means of putrescine levels and spermidine:spermine ratio were compared between the placebo group and each treatment group at 12 and 15 months. At 12 months, the geometric mean of putrescine in the placebo group was 8.32 times greater than the geometric mean of putrescine in the 0.40 dose group (95% confidence intervals (CI) = 1.50-46.2; p = 0.02) and geometric mean of the spermidine:spermine ratio was 1.63 times greater in the placebo group as compared to the 0.40 dose group (95% CI = 1.26-2.13; p = 0.001). Other dose groups did not demonstrate statistically significant differences in polyamine measures at 12 months, as compared to the placebo group. In all dose groups, including the placebo, putrescine measures increased from 12 to 15 months. At the 3-month follow-up (15 months), there was no significant difference in polyamine measures between each dose group and the placebo group. Our findings suggest that 0.40 g/sq m/day of DFMO was effective in lowering the putrescine and spermidine:spermine at 12 month-period as compared to the placebo group; however, this decrease was not sustained at 3 months post-treatment. Future trials are necessary to clarify whether DFMO treatment is effective in various doses, whether treatment benefit can be gained through other DFMO-based therapeutic regimens, and whether therapy with DFMO at the 0.40 dose level would be acceptable as an on-going therapy for the secondary prevention of colon cancer.

Comment [A1]: Somewhere in here I would mention the dropout—because there was somewhat of a trend over dose, this becomes a relevant result. The dropout could be biasing, if it were related to polyamine level. In any case the fact that it might be related to toxicity is very much of interes.

Comment [A2]: One of Koch's postulates is illustration of a dose-response. Hence, it is very much of interest to provide estimates, even when there is no statistical significance. (I note that there are many mechanisms that would affect our precision across dose groups as well, including the possibility of differing sensitivity to the DFMO dose across individuals. Hence there might be a very good trend in the mean, but a loss of precision precluded stat signif. The bottom line: Do not focus exclusively on stat stignif.)

Comment [A3]: Was there rebound? This would be of interst to talk about, so (again) reporting estimates and CI for even nonsignificant results is of interest.

Background

Colon cancer is the second leading cause of cancer deaths in the U.S. The overall age-adjusted incidence rate was 38.7 cases per 100,000 population in 2001-03.[1] Colon polyps result from excessive cell growth in the colon lining and are potential precursors of colon cancer. This excessive cell growth is believed to be stimulated by organic compounds called polyamines, with greater polyamine production occurring in growing cells than nongrowing cells. In normal cells a biochemical pathway involving polyamine production converts ornithine to putrescine via the enzyme, ornithine decarboxylase. This is followed by a rapid conversion of putrescine to spermidine. Spermidine is then converted to spermine.

[1] U.S. Centers for Disease Control and Prevention, National Center for Health Statistics, National Program of Cancer Registries, http://apps.nccd.cdc.gov/uscs/, accessed November 27, 2007.

Difluoro Methyl Ornithine (DFMO) is a pharmacological agent undergoing clinical trials for the secondary prevention of colon cancer in patients with a history of colon polyps. Previous studies using animal models have shown that the administration of DFMO in the presence of colon cancer decreases both polyamine production and the growth of cancer cells. Studies which introduced both DFMO and polyamines showed no such reversal of cancer growth, confirming that polyamines are a causal agent and not simply a byproduct of cancer cell growth. DFMO has also been shown to block the synthesis of polyamines in laboratory studies. The mechanism by which DFMO reduces polyamine production is believed to occur by inhibiting the enzyme, ornithine decarboxylase. It is believed that pathways other than that involving ornithine decarboxylase may contribute to spermine production. In addition, previous studies reported a decreased ratio of spermidine to spermine in response to blocking ornithine decarboxylase. Two outcome measures of treatment effect, believed to be the best markers of polyamine inhibition, were considered in this analysis: (1) the level of putrescine and (2) the ratio of spermidine to spermine (spermidine: spermine).

There is little known about normal levels of polyamines in humans. Polyamines are involved in growth of both normal and cancer cells, and it is unclear whether excessive inhibition of polyamines may be harmful. It is also unknown what magnitude of reduction in polyamines is necessary to produce clinically meaningful benefit for either treatment of existing colon cancer or secondary prevention of colon cancer. Previous studies of DFMO as an anti-neoplastic agent involved much higher doses of DFMO and provided evidence of ototoxicity. Because cells in normal colon tissue survive about 10 days before being shed, it is important to assure that any chemical treatment to suppress cell growth in colon tissue can achieve a sustained effect over time with minimal adverse effects.

Questions of Interest

1. Do any DFMO dose groups demonstrate decreases in polyamines at the end of the 12-month treatment period, as compared to placebo group?
2. Assuming that higher doses of DFMO may confer adverse effects, what is the lowest dose at which we see a significant difference in polyamines when compared to the placebo group?
3. Do polyamine measures tend to remain constant, increase or decrease after treatment has stopped?
4. Do any DFMO dose groups demonstrate sustained decreases in polyamines 3 months after cessation of treatment, as compared to placebo group?

Sources of the Data

A total of 114 volunteers at high risk of developing colon cancer, based on a history of colon polyps, participated in a randomized double-blind, placebo-controlled Phase IIb clinical trial to test the effect of DFMO on polyamine levels. The participants included were 97 males (85.1%) and 17 females (14.9%). In the trial, participants were randomized to one of four DFMO dose groups. The dose groups were for 0 (placebo), 0.075, 0.20, and 0.40 g/sq m/day of DFMO over a 12-month period. Colon biopsies were obtained at randomization prior to DFMO treatment, at 6 and 12 months (during the

treatment phase), and 15 months (3 months after completion of treatment). The biopsied colon tissue was used to measure levels of putrescine, spermidine, and spermine (µmol/mg protein). Age and gender of subjects were also assessed.

Statistical Methods

Descriptive statistics were used to characterize the four dose groups of the study participants with a history of colon polyps in this trial. The mean and standard deviations (SD) for age and the proportion of female participants by each dose group was calculated. Because polyamine levels are reflective of underlying cell growth rates, changes in these growth rates could be expected to produce non-linear effects on polyamine measures. This analysis used log-transformed values for the polyamine measures and relied upon comparison of geometric means of the measures in order to best handle this possible non-linear relationship. In general, the variables for putrescine, spermidine and spermine levels were not normally distributed, with a tendency to be right-skewed. The use of the geometric mean summary measure for statistical inference in this analysis tends to downweight these potentially influential observations. Because zero values for the 3 types of polyamines were assumed to represent the lower limit of detectability for these laboratory measures, rather than true zeros, zero values were replaced with the midpoint of zero and the minimum observed value for each polyamine measure. This replacement was done for 26 measurements for putrescine (replaced with 0.001 µmol/mg protein) and for 2 measurements each for spermidine and spermine (replaced with 0.1465 and 0.7275 µmol/mg protein, respectively). Our analyses are based on intent-to-treat analysis and no observation is excluded other than missing observations.

Comment [A4]: Very nicely written to show the fact that this decision was based on *a priori* considerations.

Comment [A5]: very nicely written

This analysis conducted two-way comparisons of geometric means between each dose group and the placebo group for the two outcome variables of interest at 12 and 15 months, by using t-test with the assumption of unequal variances. All comparisons of geometric means were tested against the null hypothesis of equality of geometric means, as a ratio equal to 1.0. The ratio of geometric means of putrescine and of spermidine:spermine in the placebo versus each dose group, the corresponding 95% confidence intervals (95% CI) for the ratio of geometric means, and the two-sided p-values were provided. No correction was made for multiple comparisons.

Comment [A6]: here in the methods it is probably good to go ahead and say "t tests on the log transformed measurements".

Comment [A7]: good to note

This analysis considered outcome measures in isolation of the baseline values, since the outcome measures were quite comparable at baseline across the randomized groups. The correlation between baseline and subsequent polyamine measures was not high within each dose group; correlation coefficients for putrescine ranged from -0.10 to 0.31 when baseline measures were compared to 12- and 15-month measures, and correlation coefficients for spermidine:spermine ranged from -0.12 to 0.24 when baseline measures were compared to 12- and 15-month measures. Therefore, an analysis using the difference in outcomes from baseline would have meant higher standard error estimates and decreased precision. For the spermidine:spermine measure, all inference was confined to this ratio measure, and not to the separate component measures (spermidine and spermine). All of the analyses were performed using Stata version 10.0 (StatsCorp, College Station, TX).

Comment [A8]: This decision should have been made *a priori* as well. It would not hurt here to point out these results and to comment on the fact that your prior guess was a good one.

Comment [A9]: Some justification for using this ratio would be nice. Use either prior publications of this measure or the fact that it is in some sense an internal control.

Results

Characteristics of the Study Participants

A total of 114 participants were randomized into either the placebo group or one of 3 treatment groups: placebo, 0.075, 0.20, and 0.40 g/sq m/day of DFMO. The number of subjects at baseline in each dose group varied, with 32, 29, 25, and 28 subjects in the placebo, 0.075, 0.20, and 0.40 g/sq m/day dose groups, respectively (**Table 1**). Over time, the number of subjects in every group decreased; however, the drop-out rate was greatest in the highest dose group. At 15 months, drop-out rates were 15.6% (5 subjects), 10.3% (3 subjects), 16.0% (4 subjects), and 35.7% (10 subjects) in the placebo, 0.075, 0.20, and 0.40 g/sq m/day dose groups, respectively. The mean age of participants was similar across dose groups. There were more males than females in all dose groups; the proportion of females ranged from zero in the 0.20 dose group to 21.4% in the 0.40 dose group. Baseline polyamine levels were similar across groups.

Comment [A10]: Very important to note

Trend of Polyamine Measures over Time

In the placebo group, the average putrescine level increased steadily over time, whereas spermine decreased steadily and spermidine fluctuated over time (**Figure 1**). In each of the DFMO dose groups, the trend was one of a notable decline in average putrescine measures at 6 months and then a steady increase thereafter in the 12- and 15- month measures. The average spermidine level generally decreased in each dose groups over time through 12 months, and then increased somewhat at 15 months. Spermine levels decreased through 15 months in all dose groups. As for the spermine:spermidine measure, all DFMO dose groups showed a decrease between baseline and 12 months, whereas the placebo group showed an increase in this measure during this time period (**Table 1**). At 15 months, all DFMO dose groups exhibited increases from 12 to 15 months, whereas the placebo and 0.40 dose groups had lower ratios as compared to their respective baseline values.

Placebo versus DFMO Dose Groups at 12 months

At the end of the treatment phase at 12 months, the geometric mean of putrescine in the placebo group was 8.32 times higher than in the 0.40 dose group (95% confidence intervals (CI) = 1.50-46.2; p = 0.02) (**Table 2**). In contrast, there was no statistically significant treatment effect on putrescine levels in the 0.075 nor the 0.20 dose group, as measured by the ratio of geometric means with a ratio of 1.0 representing the null hypothesis of equality of geometric means. The spermidine:spermine was 1.37 times greater in the placebo group than the 0.075 dose group (95% CI = 1.08-1.75; p = 0.01) and 1.63 times greater in the placebo group than the 0.40 dose group (95% CI = 1.26-2.13; p =0.001). While not statistically significant, the spermidine:spermine measure was also greater in the placebo group than the 0.20 dose group (point estimate for placebo to 0.20 dose geometric mean ratio=1.49, 95%CI = 0.99-2.23; p = 0.06). Hence, the only dose group that demonstrated a significant treatment effect as measured by declines both in putrescine level and spermidine:spermine compared to placebo was the 0.4 dose group.

Comment [A11]: The general trend in the estimates would be of interest even in the absence of stat signif

Placebo versus Dose Groups at 15 months

At 15 months, there was no statistically significant enduring treatment effect on either putrescine levels or spermidine:spermine in any of the DFMO dose groups when

compared to the placebo group, as measured by the ratio of geometric means with a ratio of 1.0 representing the null hypothesis of equality of geometric means (**Table 2**).

Discussion

In this clinical trial of the effect of DFMO on polyamines in colon cells, lower levels of putrescine and the ratio of spermidine to spermine in the 0.40 dose group than the placebo group were observed after the 12-month treatment period. This suggests that the 0.40 g/sq m/day of DFMO was more effective in decreasing the putrescine and the spermidine to spermine ratio at 12 month-period than the placebo group and only this highest dose group demonstrated statistically significantly lower levels. However, at the 15-month time period, 3 months after cessation of DFMO treatment, neither the 0.40 dose group nor the lower dose groups demonstrated a significant treatment effect upon polyamine levels as compared to the placebo group. This suggests that any inhibitory effect of the highest dose of DFMO on polyamine production was not sustained once subjects stopped treatment. In all treatment groups, polyamine measures at 15 months tended to increase above the average baseline and 12 month measures.

Strengths of this trial included use of a randomized double-blind, placebo-controlled design with follow-up (12 months on treatment plus 3 months of follow-up after cessation of treatment), providing evidence about the possible effectiveness of DFMO in reducing polyamine levels than previous studies. In addition, this trial pre-assigned the DFMO dose rather than adjusting it during the trial.

An important limitation in this trial was that it had a small sample size combined with differential drop-out rates among participants across dose groups. The drop-out rate was lower in the placebo, 0.075 and 0.20 dose groups (15.6%, 10.3%, and 16.0%), compared with the 0.40 dose group (35.7%). It is possible that adverse effects, such as treatment toxicity, resulted in higher drop-out rate in the 0.40 dose group. In examining the descriptive statistics, there is some suggestion that drop-outs from the placebo group were older than those who remained in the trial, whereas drop-outs from the three DFMO dose groups more closely match the original age distribution. Because no measures were taken related to toxicity of DFMO treatment or other possible causes of drop-out in the trial, it is difficult to evaluate the assumption that drop-out in this study represented non-informative censoring. It is possible that drop-outs represented either those in whom polyamine declines would have been greatest (leading to an under-estimate of the treatment effect), or those in whom declines would have been minimal (leading to an over-estimate of the treatment effect).

The sex distribution of the participants does not reflect the population at risk for colon cancer, whereas the incidence of colon cancer in males is only moderately higher than in females (age-adjusted incidence of 44.2 cases per 100,000 in men versus 39.6 cases per 100,000 in women in 2001-03).[2] Among the 114 study subjects, only 14.9% were female. Although higher variability in polyamine measures was noted at baseline, further subgroup analysis by sex and age group was not possible due to a small sample size and

Comment [A12]: It is just as much interest to see the CI here. We are interested in knowing whether the treatement effect persists, whether the levels return to normal, or whether there might even be "rebound". Only the estimates and CI can distinguish these from lack of precision. DO NOT PLACE TOO MUCH IMPORTANCE ON STAT SIGNIFICANCE. ESTIMATES AND CI ALWAYS MATTER.

Comment [A13]: I would tend to lead off with a discussion of the aspect that is potentially most biasing: The loss of follow-up that appears to be greatest at the highest doses.could be related to polyamine levels.

Comment [A14]: This is not censoring. Censoring is a very special kind of incomplete data. (Perhaps the zero polyamine levels represent left-censoring. You treated it that way.)

Comment [A15]: Very good comments. As noted, I would put this paragraph first.

[2] U.S. Centers for Disease Control and Prevention, National Center for Health Statistics, National Program of Cancer Registries, http://apps.nccd.cdc.gov/uscs/, accessed November 27, 2007.

disproportionate number of males to females. The small sample size limited our ability to assess sex or age effect on polyamines or to conduct a subgroup analysis by sex or age group.

Finally, without *a priori* information about what levels of polyamine decrease might translate into clinical benefit and without clinical endpoints other than polyamines assessed in this study, we cannot draw conclusions about whether the treatment effects observed in this study were clinically meaningful.

Comment [A16]: good

Conclusion: This trial demonstrated that the DFMO treatment of 0.40 g/sq m/day, as the highest dose tested in this trial, was more effective in lowering putrescine level and spermidine:spermine than the placebo group after the 12-month treatment period. These lower levels were not sustained at the 15-month time period, 3 months after cessation of treatment. The high drop-out rate in the 0.40 dose group suggests that there may be significant adverse effects of treatment at that dose. Future trials with larger sample sizes are necessary to clarify whether DFMO treatment is effective in various doses, whether treatment benefit can be gained through other DFMO-based therapeutic regimens, and whether therapy with DFMO at the 0.40 dose level would be acceptable as an on-going therapy for the secondary prevention of colon cancer. Such trials could help to elucidate an optimal use of DFMO treatment to achieve sustained, but not excessive, suppression of polyamines in patients at high risk of colon cancer.

Table 1. Age, Sex, and Polyamine Levels of the Study Participants by Dose Group*

Dose (g/sq m/day)	0		0.075		0.20		0.40	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
age	32	65.9 ± 8.51	28	61.3 ± 7.69	25	62.8 ± 8.28	28	63.9 ± 7.81
female (%)	6	18.8	5	17.2	0	0	6	21.4
Putrescine (μmol/mg protein)								
Baseline	32	0.66 ± 0.44	29	0.65 ± 0.52	25	0.61 ± 0.42	28	0.65 ± 0.57
6 months	30	1.06 ± 1.59	28	0.47 ± 0.27	23	0.45 ± 0.54	25	0.33 ± 0.43
12 months	28	1.16 ± 0.83	26	1.08 ± 1.03	21	0.80 ± 0.79	20	0.88 ± 1.42
15 months	27	1.54 ± 1.77	26	1.19 ± 0.89	21	1.00 ± 0.71	18	1.18 ± 0.83
Spermidine (μmol/mg protein)								
Baseline	32	3.26 ± 1.45	29	3.47 ± 1.55	25	3.35 ± 1.33	28	3.56 ± 1.88
6 months	30	3.37 ± 1.53	28	2.64 ± 0.89	23	2.58 ± 1.64	25	2.68 ± 1.43
12 months	28	3.26 ± 1.31	26	2.92 ± 0.99	21	2.71 ± 1.40	20	1.95 ± 0.80
15 months	27	2.69 ± 0.93	26	2.95 ± 0.99	21	2.98 ± 1.90	18	2.70 ± 1.87
Spermine (μmol/mg protein)								
Baseline	32	8.22 ± 5.54	29	8.43 ± 5.86	25	9.03 ± 7.04	28	8.08 ± 5.50
6 months	30	7.34 ± 2.71	28	8.07 ± 2.49	23	7.18 ± 2.48	25	8.04 ± 3.95
12 months	28	6.55 ± 3.59	26	7.75 ± 3.12	21	7.16 ± 3.15	20	5.93 ± 2.58
15 months	27	6.39 ± 2.45	26	6.69 ± 3.32	21	6.08 ± 3.56	18	6.42 ± 2.53
Spermidine: Spermine ratio								
Baseline	32	0.464 ± 0.228	29	0.459 ± 0.217	25	0.456 ± 0.315	28	0.478 ± 0.243
6 months	30	0.505 ± 0.270	28	0.343 ± 0.123	23	0.400 ± 0.333	25	0.446 ± 0.440
12 months	28	0.619 ± 0.432	26	0.410 ± 0.140	21	0.447 ± 0.277	19	0.356 ± 0.141
15 months	27	0.452 ± 0.162	25	0.529 ± 0.267	21	0.677 ± 0.502	18	0.456 ± 0.155

*All the values presented here are original values without the replacement or transformation

Comment [A17]: It would have been best to include min and max for the polyamine levels. There may be questions about individual toxicities.

Table 2. Comparison of Putrescine and the Ratio of Spermidine to Spermine in the Placebo with the DFMO Treatment Groups at 12 and 15 Months

	Comparison group (dose g/sq m/day)	Geometric mean in Placebo	Geometric mean in Comparison Group	Ratio in geometric means (placebo: treatment group)	Lower 95% CI*	Upper 95% CI*	P-value**
Putrescine (μmol/mg protein)							
12 months	0.075	0.61	0.71	0.87	0.37	2.01	0.74
	0.20	0.61	0.42	1.46	0.52	4.12	0.47
	0.40	0.61	0.07	8.32	1.50	46.2	0.02
15 months	0.075	0.98	0.78	1.26	0.63	2.50	0.50
	0.20	0.98	0.63	1.56	0.70	3.46	0.26
	0.40	0.98	0.96	1.02	0.64	1.64	0.92
Spermidine: Spermine							
12 months	0.075	0.53	0.39	1.37	1.08	1.75	0.01
	0.20	0.53	0.36	1.49	0.99	2.23	0.06
	0.40	0.53	0.32	1.63	1.26	2.13	0.001
15 months	0.075	0.43	0.46	0.93	0.74	1.18	0.56
	0.20	0.43	0.56	0.76	0.57	1.03	0.08
	0.40	0.43	0.43	0.99	0.80	1.22	0.91

*The corresponding lower and upper 95% confidence intervals (CI) of the ratio in geometric means are presented.
**All the significance testing is based on two-sided and log-transformed values

Figure 1. Trends of Polyamine Levels (µmol/mg protein) Over Time in the Placebo and the DFMO Treatment Groups