Group 4

Effect of α- difluromethylornithine administration on polyamine levels in patients with adenomatous polyps: a phase IIb trial.

Summary

Background: Colon cancer is a common malignancy with high mortality. To date no successful strategies have been identified to prevent colon cancer. One potential target is inhibition of polyamine synthesis, a protein necessary for colonic cell replication and proliferation.

Objective: We designed a phase IIb randomized double-blind placebo controlled trial to test the effects of α-difluromethylornithine (DFMO) administration at different doses on production of polyamines.

Methods: We randomly assigned 114 patients with a history of resected colon polyps to placebo or DFMO at a dose of 0.075, 0.2 or 0.4 g/m^2/day. Patients were treated for 12 months and underwent colonoscopy at entry, 6, 12 and 15 months after enrollment. Primary endpoints were spermidine to spermine ratio as well as polyamine levels at 6 and 12 months and at 15 months. Two sample t-tests with unequal variance were used to estimate the difference in mean polyamine levels between the highest DFMO dose group and the placebo group.

Results: Treatment with DFMO at 0.4 g/m^2/day resulted in decreased spermidine to spermine ratio at 12 months (0.612, 95% CI: 0.469 to 0.796; *p=0.0005*) but not at 6 months (0.753, 95% CI: 0.538 to 1.055; *p=0.0972*). Putrescine levels (0.120 µmol/mg, 95% CI: 0.022 to 0.668; *p=0.017*) and spermidine levels (0.569 µmol/mg, 95% CI: 0.404 to 0.802; *p=0.*002) were also decreased at 12 months. This ratio returned to normal 3 months after discontinuing DFMO (1.013, 95% CI: 0.819 to 1.252, *p=0.91*).

Conclusions: Our results suggest that DFMO administration at 0.4 g/m^2/day decreases the mean polyamine levels of putrescine and the spermidine to spermine ratio and that with cessation of therapy that polyamine levels return to normal levels. Similar trends were observed in all DFMO dose groups.

Comment [A1]: A very good summary

Comment [A2]: Could perhaps include age range and sex distribution here.

Comment [A3]: Comment on study dropout?

Comment [A4]: I think these are ratios, not absolute levels.

Comment [A5]: Summary statement about other doses, 6 month measurements might be useful here.

Comment [A6]: within 3 months

Background

Colon cancer is common in the United States[1] and worldwide[2] and the incidence continues to increase[3] while mortality remains high.[4, 5] Unlike other malignancies, colon cancer is associated with a precancerous lesion, an adenomatous polyp, which can be detected with screening and removed via colonoscopy.[6] Individuals with polyps are at increased risk of developing invasive carcinoma and have become a target of interest for colon cancer prevention. Current prevention efforts have evaluated the efficacy of diet, exercise, non-steroidal anti-inflammatory and experimental medications without conclusive results.[7-10]

An alternate approach to preventing colon cancer is to inhibit polyamines. These novel proteins (putrescine, spermidine, and spermine) are required for growth of normal and neoplastic cells although their actual mechanism remains unknown.[11] alpha-difluromethylornithine (DFMO), an inhibitor of polyamine synthesis, has been shown to decrease cancer cell replication in cell culture and animal models, and decreases the production of polyamines.[12-16] DFMO inhibits the conversion of ornithine to

Comment [A7]: alternative

putrescine by blocking the enzyme ornithine decarboxylase. Normally putrescine is converted rapidly to spermidine which is then converted to spermine. Prior studies have shown the DFMO tends to decrease putrescine levels without decreasing spermine levels although a decrease in spermidine to spermine ratio has been demonstrated suggesting an alternate pathway for spermine production.[17] DFMO has been shown in recent phase I and phase II trials to be safe in large doses with minimal side effects.[18, 19]

Comment [A8]: "alternate" is correct here

Little is known about the effects of DFMO administration in patients at risk for colon cancer and how polyamine levels change in response to long term administration of DFMO. We sought to evaluate the effects of DFMO administration in individuals with a history of colonic polyps.

Questions of Interest

The primary question of interest is whether treatment with DFMO results in decreased levels of the polyamines putrescine, spermine and spermidine, as well as the ratio of spermidine to spermine to correct for polyamine production outside of the ornithine decarboxylase pathway. Additionally, we sought to determine if a reduction persisted over time, whether levels continued to decrease with continued administration of DFMO, and whether ploymine levels returned to normal after ending treatment.

Source

The study was a Phase IIb randomized, double blind placebo controlled trial conducted at the University of California, Irvine. The study enrolled 114 subjects, comprised of 17 females and 97 males, with a prior history of colon polyps resected by colonoscopy. Subjects were randomly assigned to receive placebo or DFMO at a dose of 0.075, 0.2, or 0.4 g/m^2/day for 12 months. Putrescine, spermidine, and spermine levels (measured in µmol/ mg of protein) were obtained from colon biopsies at the time of enrollment, after 6 and 12 months of treatment, and 3 months after completing treatment. Additionally, information on patient age, sex and dose group was obtained.

Statistical Analysis

Missing data were excluded from descriptive statistics and statistical analysis. Summary measures include the number of missing observations, mean and standard deviation, median (for continuous variables), minimum, and maximum. To correct for polyamine levels below the sensitivity of the assay, protein levels of zero were substituted with values half the minimum reported value. Polyamine levels were compared using geometric means as polyamine levels were log transformed to correct for heteroscedasticity with large standard deviations of polyamine levels and slight skewness in the data [Table 1]. Since correlation of polyamine levels between time points were less than 0.5, this suggests that there is little correlation between baseline and each time

Comment [A9]: I would say what that value was here.

point, therefore comparisons were made between dose groups and placebo rather than to baseline polyamine levels. Polyamine levels and spermidine to spermine ratios in each treatment group were compared to those in the placebo group at each assessment point using two sample t-tests with unequal variances. To minimize the problem of multiple comparisons, we analyzed the difference between the placebo and highest DFMO dose group at 6, 12 and 15 months. As part of a post-hoc analysis we analyzed differences in between placebo and other dose groups as well. As this was a randomized trial, we did not consider confounding or effect modifiers.

The estimated mean difference, 95% confidence intervals, as well as two-sided p-values are provided. A p-value of less than 0.05 was considered to be statistically significant. All statistical analyses were performed using STATA version 10 for Windows (StataCorp LP, College Station, TX, USA).

Comment [A10]: You would make this decision prior to looking at the data. This wording is a little bit unclear. Perhaps say " comparisons were made between dose groups with respect to the polyamine levels without adjustment for the baseline values".

Comment [A11]: Why use the spd :spm ratio? (Prior studies show little change in spm, and hence we can use that value as an "internal control" for changes in the spd levels. This would control for variation in the amount of colonic mucosa in the biopsy, etc.)

Comment [A12]: OK, but I might have stated it as "our primary endpoint was defined as …"

Comment [A13]: I thought you said you were using geometric means

Results

Baseline characteristics for patients in each treatment group are shown in Table 1. Baseline polyamine levels were similar in all treatment groups. Patient age ranged from 45 to 80 years old, with approximately 20% women in each dose group, except for the 0.2 g/ m^2/day DFMO dose group where no women were assigned. There were 32 patients assigned to the placebo group, 29 to the 0.075 g/m^2/day, 25 to the 0.2 g/m^2/day, and 28 to the 0.4 g/m^2/day DFMO dose group. Plots of polyamine levels at various times versus patient age by treatment group shows evidence of heteroscedasticity and examination of the polyamine levels at the various study times reveals a slight rightward skewness in the data. These findings suggest a log transformation of the data is appropriate.

Comment [A14]: show

Comment [A15]: Statistical jargon. Put it into words you would have understood prior to taking this class.

Comment [A16]: Hopefully you made this decision prior to looking at the data. It is not incorrect to comment on the fact that these properties are consistent with the decision you made *a priori.*

The plots of spermine levels at the beginning of treatment versus age reveal four potential outliers (one in each dose group). These subjects are each approximately 65 to 70 years old, and have spermine levels between 35 and 40 µmol/mg protein.

Comment [A17]: I do not see these plots. Having said that measurements tend to be skewed, I would guess that we should be careful calling these "outliers". If you think the distn is skewed, there will tend to be stragglers. This is just an issue of connotation in a paper—"outliers" will sometimes give a mental image of aberrant values, rather than just the typical tails of a skewed distn.

With increased time on study, the number of missing observations increases with the highest number of missing values occurring in the highest DFMO dose group (0.4 g/ m^2/day). At 6 months there were 2 missing observations in the placebo group, 1 in the 0.075 g/ m^2/day, 2 in the 0.2 g/ m^2/day, and 3 in the 0.4 g/ m^2/day group. At 12 months, there were a total of 4 missing observations in the placebo group, 3 in the 0.075 g/ m^2/day, 4 in the 0.2 g/ m^2/day, and 8 in the 0.4 g/ m^2/day. At 15 months there was no further increase in missing data except for one more missing observation in the placebo group. Patients who dropped out of the study were not different from those who continued in terms of age, sex and baseline polyamine levels compared to those patients who remained in the study.

Comment [A18]: You could also try to comment on the 6 month measurements for those subjects dropping out between 6 and 12 months.

There is a general trend towards decreased geometric mean polyamine levels in all dose groups with time on drug with an increase in polyamine levels 3 months post-treatment as

seen in Table 2. As with the decrease in geometric mean polyamine levels, the ratio of spermidine to spermine tends to decrease with time in study. These trends can also be seen in Figure 1.

Administration of DFMO at 0.4 g/m^2/day resulted in decreased polyamine levels compared to placebo (Table 3). At 6 months, geometric mean putrescince level was 0.092 µmol/mg lower than the placebo group (95% CI: 0.03 to 0.286; *p=0.0002*) which was similar at 12 months into treatment (0.120 µmol/mg lower, 95% CI: 0.022 to 0.668; *p=0.017*). Spermidine level was also lower at 6 months (0.778 µmol/mg, 95% CI: 0.608 to 0.996; *p=0.047*) and 12 months (0.569 µmol/mg, 95% CI: 0.404 to 0.802; *p=0.002*). However, there was no difference in geometric mean spermine level compared to placebo at 6 months (1.033 µmol/mg, 95% CI: 0.803 to 1.329; *p=0.79*) or at 12 months (0.923 µmol/mg, 95% CI: 0.662 to 1.310; *p=0.67*). Difference in geometric mean spermidine to spermine ratio was not decreased at 6 months (0.753 µmol/mg , 95% CI: 0.538 to 1.055; *p=0.097*) but was lower at 12 months (0.612, 95% CI: 0.469 to 0.796; *p=0.0005*) of treatment with DFMO.

Three months off medication, geometric mean polyamine levels increased and there was no difference in geometric mean polyamine levels for putrescine (0.977 µmol/mg, 95% CI: 0.611 to 1.563; *p=0.92*), spermidine (1.014 µmol/mg, 95% CI: 0.822 to 1.250; *p=0.90*), spermine (1.001 µmol/mg, 95% CI: 0.782 to 1.282; *p=0.99*), or spermidine to spermine ratio (1.013, 95% CI: 0.819 to 1.252; *p=0.91*) between placebo and the 0.4 g/m^2/day group.

A similar trend can be seen in the 0.075 g/m^2/day DFMO group compared to placebo at 6 months but not at 12 months except with the spermidine to spermine ratio which is decreased at both 6 months and 12 as shown in Table 3. A marginal significant difference is seen in geometric mean polyamine levels of spermidine to spermine ratio between placebo and the 0.2 g/m^2/day dose group at 6 and 12 months, while a significant difference is observed in putrescine and spermidine levels at 6 months.

Discussion

The main goal of this study was to determine whether treatment with DFMO results in a decreased ratio of spermidine to spermine as well as levels of the polyamines putrescine, spermidine and spermine. The results suggest that treatment with DFMO at a dose of 0.4 g/m^2/day does decrease putrescine and spermidine levels as early as 6 months after the beginning of treatment, although this effect is not observed in spermine levels. A reduction in the ratio of spermidine to spermine is also seen, however this effect is only seen 12 months into treatment. This suggests that increased time on DFMO results in further suppression of polyamine levels. Polyamine levels and the ratio of spermidine to spermine appear to have returned to the same level as the placebo group 3 months after

Comment [A19]: I presume you are backtransforming the difference in mean log polyamine values. That means you are talking about the ratio of geometric means. Your wording needs some work.: There are no units for a ratio.

Comment [A20]: "At 6 months, the geometric mean putrescine level in the 0.4 dose group was only 0.092 as high as that in the placebo group…" or "At 6 months the geometric mean putrescine level in the 0.4 dose group was 91.8% lower than that in the placebo group…"

Comment [A21]: Ratio

Comment [A22]: WAY too deterministic. 0.753 is certainly less than 1, so the estimate certainly was decreased. I will grant you that the difference was not statistically significant, but do NOT confuse lack of statistical significance with proof of equality.

Comment [A23]: With the ratios so close to 1, you are more justified in claiming that the observed ratios were indicative of no difference in the geometric means. Personally, however, I would state "no statistically significant differences"

Comment [A24]: At this point, I would be far more interested in the trends of the estimates, rather than trends in statistical significance. You stated that these were exploratory, because you were trying to avoid multiple comparison problems. To that end, all the p values are difficult to interpret. The estimates are not. So I would have talked about the estimates being decreased. And then I might have added which comparisons were statistically significant when not adjusting for the multiple comparisons.

But, again, I point out that you seem to be regarding that lack of statistically significant differences means no differences. That is incorrect.

Comment [A25]: I would first mention the study dropout, because the relatively large amount of missing data in the 0.4 group may bring into question the validity of the analysis—especially if due to toxicity and that toxicity were associated with the lowest values. (Of course, we don't have the values of the patients who dropped out, so all we can do is speculate.)

the end of DFMO treatment, which suggests that the effects of DFMO treatment are transitory. In general, these findings are apparent for all three doses of DFMO. Although the 6 month and 12 month suppressions were significant in both putrescine and spermidine in the highest DFMO dose group, we may be more inclined to believe the spermidine to spermine ratio since it provides a less variable measure of suppression.

Comment [A26]: why would it do this? explain (see above for the reasoning that I would use)

To minimize the possibility of multiple comparisons effecting our results we limited our primary analysis to the high dose group (0.4 g/m^2/day) believing that we would be more likely to see an effect at this dose. We did analyze the effects of DFMO administration at lower doses post-hoc to evaluate for a trend in response. We found that treatment with the lowest dose resulted in significant reductions in mean polyamine levels, while the mid-range dose resulted in marginally significant reductions in mean polyamine levels. There are several possibilities to explain this. The first is that the sample sizes were quite small, and hence perhaps the effect was not detected in the 0.2 g/m^2/day DFMO dose group. Another possibility is that there is a sex difference that accounts for the differences seen since there were no women in the 0.2 g/m^2/day dose group. This suggests a possibility of a sex effect, however our study design did not allow us to evaluate this observation. Future studies might involve a regression analysis that includes sex as a covariate.

Comment [A27]: affecting

There were more patients lost to follow-up in the highest DFMO dose group, which suggests the possibility that patients were unable to tolerate the medication and its effects.

Comment [A28]: Yes. And that toxicity could be related to polyamine levels (in which case our analyses might be biased) or not.

Our study suggests that administration of DFMO dose result in decreased polyamine levels. Future studies will need to address the questions of medication tolerance and whether DFMO results in decreased incidence of colonic polyps or cancers.

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Table 1: Descriptive statistics for baseline patient characteristics

	Placebo Group (N = 32)						Dose = 0.075 g/sq m/day (N=29)					
	# miss	Mean	SD	Min	P50	Max	# miss	Mean	SD	Min	P50	Max
Sex %												
Female	0	18.8					0	17.2				
Male	0	81.2					0	82.8				
Age	0	65.870	8.507	45.460	66.370	77.180	1	61.350	7.692	47.800	61.430	76.850
Putrescine	0	0.665	0.441	0.061	0.569	1.975	0	0.655	0.522	0.009	0.537	2.588
Spermidine	0	3.262	1.451	1.398	2.934	7.054	0	3.474	1.551	1.509	2.910	7.017
Spermine	0	8.215	5.536	1.455	7.516	35.550	0	8.429	5.860	4.130	7.318	37.667
	Dose = 0.2 g/sq m/day (N = 25)						**Dose = 0.4 g/sq m/day (N = 28)**					
	# miss	Mean	SD	Min	P50	Max	# miss	Mean	SD	Min	P50	Max
Sex %												
Female	0	0					0	21.4				
Male	0	100					0	78.6				
Age	0	62.840	8.281	45.430	63.690	77.580	0	63.870	7.808	48.520	64.970	80.970
Putrescine	0	0.612	0.418	0.000	0.595	1.964	0	0.654	0.570	0.000	0.596	2.300
Spermidine	0	3.350	1.329	1.700	2.924	6.218	0	3.565	1.885	0.660	3.084	7.600
Spermine	0	9.026	7.038	2.537	7.527	41.684	0	8.083	5.496	2.277	6.806	34.044

Polyamine levels are measured in µmol/mg.

Table 2: Geometric mean polyamine levels by dose group over time

	Dose of DFMO							
	Placebo		0.075		0.2		0.4	
	Mean	SD (min, max)	Mean	SD (min, max)	Mean	SD (min, max)	Mean	SD (min, max)
Putrescine								
0 mo	0.519	2.19 (0.06, 1.97)	0.460	2.86 (.009, 2.58)	0.450	5.77 (.001, 1.96)	0.523	9.06 (.001, 2.3)
6 mo	0.712	2.27 (0.05, 9.14)	0.249	7.29 (0.001, 1.05)	0.234	4.99 (0.001, 2.43)	0.066	14.36 (0.001, 1.73)
12 mo	0.614	6.91 (0.001, 3.18)	0.707	2.78 (0.04, 4.29)	0.421	5.30 (0.001, 3.21)	0.074	29.04 (0.001, 5.48)
15 mo	0.981	2.45 (0.30, 6.51)	0.780	4.45 (0.001, 3.53)	0.629	4.96 (0.001, 2.59)	0.958	1.94 (0.23, 3.20)
Spermidine								
0 mo	2.972	1.55 (1.4, 7.05)	3.168	1.55 (1.51, 7.02)	3.128	1.45 (1.7, 6.22)	3.105	1.74 (.66, 7.6)
6 mo	3.070	1.54 (1.51, 6.91)	2.509	1.38 (1.39, 5.12)	2.229	1.69 (1.07, 7.84)	2.389	1.60 (1.06, 6.34)
12 mo	3.008	1.51 (1.01, 5.91)	2.750	1.44 (1.35, 4.92)	2.325	1.90 (0.29, 6.45)	1.711	1.94 (0.15, 3.42)
15 mo	2.536	1.42 (1.25, 4.62)	2.643	1.91 (0.15, 4.83)	2.852	1.35 (1.81, 4.81)	2.571	1.40 (1.29, 4.47)
Spermine								
0 mo	7.171	1.68 (1.46, 35.55)	7.614	1.47 (4.13, 37.67)	7.937	1.56 (2.54, 41.68)	7.188	1.55 (2.27, 34.04
6 mo	6.878	1.45 (3.31, 14.39)	7.745	1.34 (4.60, 15.67)	6.705	1.49 (2.34, 12.13)	7.107	1.69 (2.76, 17.20)
12 mo	5.671	1.73 (2.32, 14.55)	7.115	1.50 (3.15, 14.13)	6.520	1.35 (2.96, 13.83)	5.274	1.81 (1.73, 10.66)
15 mo	5.950	1.48 (2.83, 12.05)	5.79	1.87 (0.73, 12.38)	5.110	1.85 (1.93, 12.38)	5.957	1.50 (2.53, 11.52)
Spermidine/Spermine								
0 mo	0.414	1.62 (0.12, 1.16)	0.416	1.57 (0.13, 1.11)	0.394	1.67 (0.12, 1.73)	0.432	1.55 (0.2, 1.16)
6 mo	0.446	1.65 (0.14, 1.10)	0.324	1.40 (0.20, 0.64)	0.332	1.74 (0.17, 1.72)	0.336	2.02 (0.16, 2.20)
12 mo	0.530	1.69 (0.21, 2.07)	0.387	1.43 (0.19, 0.73)	0.357	2.22 (0.03, 1.03)	0.324	1.47 (0.18, 0.76)
15 mo	0.426	1.41 (0.24, 0.97)	0.457	1.65 (0.20, 1.21)	0.558	1.82 (0.25, 2.18)	0.432	1.41 (0.22, 0.75)

Polyamine levels are measured in µmol/mg.

Comment [A29]: Mean, or geometric mean? It would be okay to present Mean in this table of descriptive statistics and then just use GM in the table of inferential statistics.

Comment [A30]: SD or SD of log transformed values? It was very important to present the min and max to allow readers to consider the possibility of toxic levels in some individuals (even though we don't know what those might be).

Table 3: Estimated difference in polyamine levels by treatment group compared to placebo

Dose of DFMO (g/m^2/day)	0.075		0.2		0.4	
	Mean Diff	95% CI (p-value)	Mean Diff	95% CI (p-value)	Mean Diff	95% CI (p-value)
Putrescine						
6 mo	0.350	0.154, 0.795 (0.0136)*	0.328	0.155, 0.694 (0.0049)*	0.092	0..03, 0.286 (0.0002)*
12 mo	1.151	0.496, 2.670 (0.7368)	0.685	0.243, 1.935 (0.4675)	0.120	0.022, 0.668 (0.0173)*
15 mo	0.795	0.400, 1.579 (0.5029)	0.641	0.289, 1.422 (0.2631)	0.977	0.611, 1.563 (0.9206)
Spermidine						
6 mo	0.817	0.669, 0.998 (0.0479)*	0.726	0.553, 0.954 (0.0226)*	0.778	0.608, 0.996 (0.0468)*
12 mo	0.914	0.740, 1.129 (0.3972)	0.773	0.558, 1.070 (0.1166)	0.569	0.404, 0.802 (0.0022)*
15 mo	1.042	0.780, 1.393 (0.7746)	1.125	0.930, 1.360 (0.2190)	1.014	0.822, 1.250 (0.8959)
Spermine						
6 mo	1.126	0.946, 1.341 (0.1778)	0.975	0.785, 1.210 (0.8139)	1.033	0.803, 1.329 (0.7940)
12 mo	1.255	0.958, 1.643 (0.0974)	1.150	0.863, 1.531 (0.3321)	0.923	0.662, 1.310 (0.6682)
15 mo	0.973	0.728, 1.300 (0.8503)	0.859	0.628, 1.175 (0.3302)	1.001	0.782, 1.282 (0.9925)
Spermidine/Spermine						
6 mo	0.726	0.581, 0.906 (0.0055)*	0.745	0.554, 1.002 (0.0515)	0.753	0.538, 1.055 (0.0972)
12 mo	0.729	0.571, 0.929 (0.0119)*	0.672	0.447, 1.010 (0.0554)	0.6117	0.469, 0.796 (0.0005)*
15 mo	1.071	0.844, 1.359 (0.5646)	1.310	0.970, 1.767 (0.0760)	1.013	0.819, 1.252 (0.9057)

* = statistically significant value
Polyamine levels are measured in μmol/mg.

Comment [A31]: ratio of geometric means, I believe

Figure 1: Spermidine to spermine ratio by dose group at each time interval