Title

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Journal

Amino acids, 33(2)

ISSN

1438-2199

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Publication Date

2007-08-30

Peer reviewed

Amino Acids (2007) 33: 189–195
DOI 10.1007/s00726-007-0515-2
Printed in The Netherlands

Amino Acids

Rationale for, and design of, a clinical trial targeting polyamine metabolism for colon cancer chemoprevention

Review Article

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Received December 7, 2006
Accepted February 1, 2007
Published online March 30, 2007;

Summary. Polyamine metabolic genes are downstream targets of several genes commonly mutated in colon adenomas and cancers. Inhibitors of ornithine decarboxylase, such as difluoromethylornithine (DFMO), and agents that stimulate polyamine acetylation and export, such as non-steroidal anti-inflammatory drugs (NSAIDS), act at least additively to arrest growth in human cell models and suppress intestinal carcinogenesis in mice. These preclinical studies provided the rationale for colon cancer prevention trials in humans. A Phase IIb clinical study comparing the combination of DFMO and the NSAID sulindac versus placebo was conducted. Endpoints were colorectal tissue polyamine and prostaglandin E2 contents and overall toxicity to participants. Participants in the Phase IIb study served as a vanguard for a randomized, placebo-controlled prospective Phase III trial of the combination of DFMO and sulindac with the primary study endpoint the prevention of colon polyps. Seventy percent of participants will have completed the three years of treatment in December 2006.

Keywords: Colon cancer – Chemoprevention – Polyamines – Difluoromethylornithine – Nonsteroidal anti-inflammatory drugs – Clinical trials

Abbreviations: APC, Adenomatous polyposis coli; COX1, cyclooxygenase 1; COX2, cyclooxygenase 2; DFMO, difluoromethylornithine; FAP, familial adenomatous polyposis; HNPCC, hereditary non-polyposis colon cancer; NSAIDS, non-steroidal anti-inflammatory drugs; ODC, ornithine decarboxylase; PPARγ, peroxisomal proliferator activated receptor γ; SAT, spermidine/spermine N^1-acetyltransferase; SNP, single nucleotide polymorphism

Signaling polyamine metabolism by genes commonly mutated in colon cancer

Recently, analysis of over 13,000 genes indicated that a subset of approximately 11 genes per tumor, that contribute to carcinogenesis, are commonly mutated in human colorectal cancers (Sjoblom et al., 2006). Two of the most commonly mutated genes in colon cancer found in this study include the adenomatous polyposis coli (*APC*) tumor suppressor gene and the *K-RAS* oncogene. The expression of several polyamine metabolic genes is affected by these signaling molecules (Gerner and Meyskens, 2004). Polyamine synthesis and levels of individual polyamines increase in colorectal cancers, compared to adjacent apparently normal mucosal tissue (Hixson et al., 1993).

One mechanism by which *APC* mutations promote tumorigenesis is to increase transcription of ornithine decarboxylase (*ODC*), via a *c-MYC* dependent process, and polyamine synthesis in human cells and $Apc^{Min/+}$ mice (Erdman et al., 1999; Fultz and Gerner, 2002). ODC enzyme activity and polyamine contents are also elevated in the apparently normal colonic mucosa of pre-symptomatic, genotype positive individuals with familial adenomatous polyposis (FAP), an inherited syndrome caused by mutations/deletions in the *APC* gene (Giardiello et al., 1997). K-RAS acts to increase cell and tissue polyamine contents by increasing ODC enzyme activity and by downregulating expression of the spermidine/spermine N^1-acetyltransferase (*SAT*) via a transcriptional mechanism involving the peroxisomal proliferator activated receptor γ (*PPARγ*) (Ignatenko et al., 2004). *SAT* encodes an enzyme initiating polyamine catabolism and export (Gerner and Meyskens, 2004). These observations provide strong rationale for targeting polyamine metabolism for

both chemoprevention and chemotherapy of human colorectal cancers.

Polyamine metabolism as a target for combination chemoprevention

Inhibitors of polyamine synthesis, such as the specific ODC inhibitor difluoromethylornithine (DFMO), suppress intestinal and colon carcinogenesis in experimental murine models (Gerner et al., 2003). Recent observations from our own studies and those of others indicate that several non-steroidal anti-inflammatory drugs (NSAIDS), which have the ability to suppress carcinogenesis in some tissues, activate SAT as part of their anticancer activity. These activation mechanisms are NSAID-specific (Babbar et al., 2006), but involve *PPAR* γ in the case of sulindac (Babbar et al., 2003). Further, we have obtained evidence suggesting that diet and genetic host factors may distinguish between individuals who will or will not benefit from specific, high priority colon cancer preventive agents. Specifically, we have found that DFMO only suppresses the development of high grade colon adenomas that form in the $Apc^{Min/+}$ mouse as a consequence of dietary supplementation of arginine at levels corresponding to arginine consumption in humans (Yerushalmi et al., 2006). These results suggest that DFMO may be most effective in reducing high risk, as suggested by high grade, adenomas while having little effect on the inhibition of low grade colon adenomas. Further, we find that the potent effect of sulindac on intestinal tumorigenesis is substantially suppressed when $Apc^{Min/+}$ mice are provided dietary putrescine at levels similar to those consumed by many humans (Ignatenko et al., 2006). While these data supported our contention that the mechanism of inhibition of intestinal carcinogenesis by sulindac involves the polyamines, these data also point out that normal dietary habits of participants in chemoprevention trials might impact the efficacy of this agent. Finally, we have described the functional significance of a single nucleotide polymorphism (SNP) in the *ODC* promoter and found that this SNP is associated in a statistically significant manner with risk of colon adenoma recurrence, especially in individuals taking aspirin (Martinez et al., 2003). The relationship between this ODC SNP and aspirin and risk of polyp recurrence has now been independently corroborated in participants of a prospective randomized trial of aspirin for colon polyp prevention (Barry et al., 2006). We speculate that the mechanism of this association involves the combined action of the +316 *ODC-A* allele-specific suppression of *ODC* transcription, by E-box repressors including MAD1, and aspirin activation of SAT and polyamine export, as depicted in Fig. 1. Consideration of all facets of polyamine metabolism, including processes affecting uptake, synthesis, catabolism and efflux, may be required to adequately target this pathway for cancer prevention and/or treatment (Basuroy and Gerner, 2006).

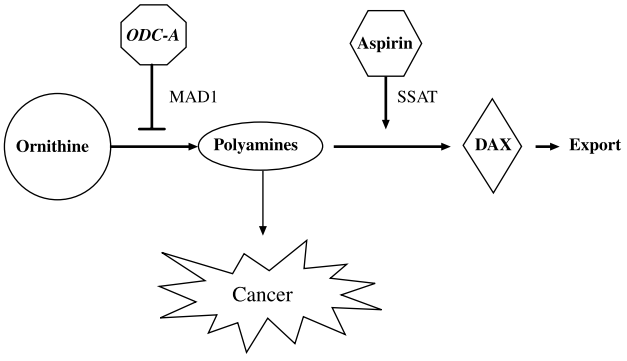

Fig. 1. Model for association of *ODC-A* allele and aspirin usage and the reduction in risk of recurrence of colon polyps. The association between the *ODC-A* allele and aspirin in individuals with lower colon polyp recurrence, as discussed in the text, may be due to lower polyamine synthesis as a consequence of MAD1-dependent suppression of *ODC* transcription and enhanced polyamine acetylation and export, as a consequence of aspirin-induced SAT acetylation

Translational studies leading to the design of a clinical trial of combined DFMO and NSAID for prevention of colon polyps

DFMO dose delivery and scheduling for chemoprevention

Prospective users of cancer chemopreventive agents will be essential healthy individuals who have not yet developed invasive cancer. Consequently, we wanted to use oral dosing as a simple and easy method of agent delivery. DFMO has a short serum half-life in humans treated with this agent (Grove et al., 1981; Love et al., 1993). However, studies had noted that the ODC enzyme inhibiting activity of DFMO was retained in cells after removal of the drug from culture medium. This result suggested metabolism of DFMO and retention of active drug by treated cells. Consequently, we conducted pilot clinical trials of DFMO in humans with prior colon polyps, who were treated for periods of one month with oral doses of DFMO. The initial study endpoints were colorectal tissue polyamine contents, which were validated in normal human volunteers (Hixson et al., 1994). We began by treating individuals with prior colon polyps with a dose of DFMO used in cancer therapeutic trials ($3\,gm/m^2/day$

for one month), and documented that this dose reduced colorectal polyamine contents, compared to pre-treatment levels in the same individuals (Boyle et al., 1992).

A unique dose de-escalation study design was then used in a phase IIa trial to determine the lowest oral dose of DFMO that was effective in reducing colorectal polyamine contents (Meyskens et al., 1994). This non-randomized trial investigated doses as low as 0.075 gm/m^2/day for one month, and found that low oral doses of DFMO apparently reduced colorectal polyamine contents, and that serum levels of DFMO did not correlate with apparent tissue levels of polyamines. The phase IIa study was followed by a prospective, placebo-controlled randomized phase IIb trial of three DFMO doses (0.075, 0.2 and 0.4 gm/m^2 per day) versus placebo for one year. This study concluded that an oral dose of 0.2 gm/m^2 DFMO per day was both effective in reducing colorectal mucosal polyamine contents and safe, in that no toxicities were observed to be elevated in this treatment group, compared to the placebo group (Meyskens et al., 1998).

Combination chemoprevention with DFMO and an NSAID

Experimental studies have shown that DFMO acts at least additively with a number of NSAIDS, including the cyclooxygenase 1 (COX1) selective agent aspirin (Li et al., 1999), the cyclooxygenase 2 (COX2) selective agent celecoxib (Zell, 2006) and non-selective inhibitors of both COX1 and COX2, including piroxicam (Rao et al., 1991) and sulindac (Lawson et al., 2000). Much of this information was either published or available to us in unpublished form in 1996, when we were considering options to extend our translational studies of DFMO in colon cancer prevention. We favored using another suitable agent in combination with DFMO, as it had been recognized by others that combinations had advantages over single agents in chemoprevention strategies (Sporn, 1980).

Based on the experimental evidence available, we decided to add an NSAID to DFMO in our prevention approach. Our choice of NSAID was influenced by the strong epidemiological, but lack of clinical trial, evidence for aspirin as a colon cancer preventive agent in humans in 1996 (Greenberg and Baron, 1996). At this same time, strong interest was developing in COX2 selective agents, as reports began to appear suggesting that COX2 may only be expressed in neoplastic gastrointestinal tissue, and consequently, be less toxic than agents such as aspirin (DuBois et al., 1996). We were skeptical of speculations that the then new COX2-selective agents would be safer than available COX1-selective or non-selective agents, as only limited clinical information was available on these agents in 1996. This lack of clinical safety data, plus the recognition that both COX1 and COX2 were likely involved in human colon carcinogenesis, caused us to look closely at non-selective agents. At that time, it was apparent that not all NSAIDS were equal in their toxicity profiles, and some reports suggested that sulindac might have less cardiovascular toxicity than other non-selective COX inhibitors (De Leeuw, 1996). Clinical evidence was also accumulating for beneficial effects of sulindac on colon polyp formation, especially in high risk individuals such as those with FAP (Giardiello et al., 1993). We confirmed that sulindac acted additively or better with DFMO to suppress growth of human colon cancer cells (Lawson et al., 2000). Subsequently, we chose sulindac as the NSAID to add to DFMO for our next round of translational studies of combination chemoprevention of colon cancer. Sulindac was effective in reducing colon polyps in FAP patients when used at a dose of 150 mg twice per day (Giardiello et al., 1996). We chose a dose of 150 mg once per day for our combination chemoprevention translational studies in an attempt to minimize potential toxicities in our essentially healthy participants in these trials.

Conversion from oral liquid to oral pill form of DFMO

Our studies up to 1998 administered DFMO orally in liquid form, and resulted in the conclusion that a dose of 0.2 gm/m^2/day DFMO was both effective in reducing colorectal mucosal polyamine contents, and was safe (Meyskens et al., 1998). We wanted to further simplify our methods of delivering chemopreventive agents, and use pills to deliver both our sulindac and DFMO doses. Sulindac was already available in pill form. Others had determined that the bioavailability of DFMO was the same in tablet and liquid forms (Carbone et al., 2000). Consequently, we chose a dose of 500 mg DFMO in tablet form, approximating the 0.2 gm/m^2/day dose for an average individual, for our combination chemoprevention trials.

Phase IIb/III clinical trial of combination DFMO and sulindac for colon cancer prevention

Our first approach to combination chemoprevention with DFMO and sulindac was to document safety of the combination over an extended period. Consequently, we proposed and conducted a prospective, randomized placebo-controlled phase IIb trial of the combination of

Table 1. Inclusion and exclusion criteria for Phase IIb/III clinical trial of DFMO + sulindac for colon polyp prevention

Inclusion criteria	Exclusion criteria
Male and female individuals, ages 40–80 with a history of one or more resected adenomas 3 mm or greater in any dimension within 5 years from study entry.	History of invasive cancer within 5 years, excepting those with adequately treated non-melanomatous skin cancer, level I (or Breslow <0.76 mm) cutaneous melanoma, Stage I cervical cancer, or CLL (Stage 0).
A screening colonoscopy must be done within 6 months of study entry and all polyps measured, removed, pathologically examined, and tissue archived.	Anticipated radiation or chemotherapy.
Individuals must have normal renal and liver function. Objective criteria would include serum creatinine must be ≤ 1.5 mg/dl, and serum bilirubin must be ≤ 2.0 mg/dl. The issue of including patients with chronic diseases is a challenging one when the underlying condition is well controlled. We have therefore left the decision up to the participant, primary care doctor, and site PIs when the objective criteria for eligibility have been met.	Personal or family history of familial polyposis or hereditary non-polyposis colon cancer.
Able to meet Southwest Oncology Group performance status criteria of 0–1, where 0 = fully active and able to carry out all pre-disease activities without restrictions (Karnofsky Scale 90–100) and 1 = restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (Karnofsky Scale 70–80).	Concomitant use of corticosteroids, nonsteroidal anti-inflammatories, or anticoagulants on a regular or predictable intermittent basis. No participants on cardiovascular prophylaxis (81 mg ASA, orally, every day or up to 350 mg twice per week) will be allowed.
Anticipated regional geographic stability over the next 36 months.	History of abnormal wound healing or repair, nor conditions that predispose to the same.
Signed informed consent approval by the local Human Subjects Committee (IRB).	Personal history of colon resection >40 cm or resection of the ileocecal valve or history of inflammatory bowel disease.
Successfully completed run-in period.	Pregnant or lactating women are not eligible. History of allergies to NSAIDs or DFMO. Documented history of gastric/duodenal ulcer within last 24 months. If potential participants have an older history than 24 months and were adequately treated, they will be considered eligible. Not currently being treated for gastric/duodenal ulcer or experiencing symptoms at study entry.

500 mg/day DFMO and 150 mg/day sulindac for 3 years. The primary study endpoints were biochemical parameters relating to the agents used, and included colorectal mucosal polyamine contents and prostaglandin E2 levels assessed prior to entry on study (baseline), and after both 12 and 36 months on study. This study was powered to accrue 200 participants at the completion of the trial in order to detect differences in these endpoints based on the results of our and others' previous trials. The study was not powered to detect a difference in polyp recurrence between placebo and treatment arms, as other chemoprevention trials to detect such differences often required more than 1000 patients (Alberts et al., 2000). Polyp recurrence was documented and scored as a secondary endpoint.

Patient entry criteria are listed in Table 1. Participants were males and females age 40–80 years, with a prior history of at least one colon polyp larger than 3 mm within 5 years of entry into the trial. All patients received a screening colonoscopy within 6 months of entry into the trial, at which time any polyps were measured, removed, examine pathologically and stored for future analyses. The minimum size of the qualifying adenomatous polyp was extensively discussed. Although no data are available that directly answer whether a 3, 5, or 10 mm polyp is inevitably, with time, on the pathway to cancer, we adopted the view that any adenomatous polyps, regardless of size, are appropriate surrogates for the carcinogenic process that leads to cancer. Exclusion criteria included invasive cancer (excepting adequately treated non-melanomatous skin cancer) within five years of study entry, severe metabolic disorders or significant acute or chronic diseases that would limit trial participation, personal or family history of FAP or hereditary non-polyposis colon cancer (HNPCC), a history of abnormal wound healing or gastric/duodenal ulcer within last 12 months, pregnancy or lactation in women, allergies to either NSAIDS or DFMO and current NSAID use, excepting aspirin doses of less than 100 mg per day. Randomization was stratified by aspirin use.

This trial commenced in 1997 and was funded by a contract from the National Cancer Institute (NCI) of the

United States of America. As this trial neared completion in 2002, we considered initiating a new trial of this combination, with polyp recurrence as the primary study endpoint. An application to the NCI was unsuccessful, in part due to the high cost of a study powered to detect small differences in polyp recurrence between treatment and placebo arms. At this time, we made a decision to propose a revised trial design that was based on at least a 50% difference between treatment and placebo arms. Our rationale was that both DFMO and sulindac were known to be potent chemopreventive agents in experimental models, and both were effective altering specific biochemical processes in human colorectal mucosa. Further, we decided that if this combination of agents only produced a therapeutic effect smaller than a 50% reduction in colon polyps, this combination therapy would not have sufficient benefit to warrant the potential risks of these agents to essentially healthy subjects. By increasing our projected treatment effect, we were able to reduce the number of participants required to observe that effect. To observe at least a 50% reduction in the number participants with recurrent colon polyps, we needed 292 participants to complete the trial. Consequently, we planned to accrue 400 participants to ensure successful completion of the study.

This trial was subsequently funded by the NCI. The study schema is shown in Fig. 2. Prior to randomization, medical histories and physical examinations were conducted. Because ototoxicity was known to be a significant side effect of one of the agents (DFMO), baseline audiometry was conducted along with a screening colonoscopy.

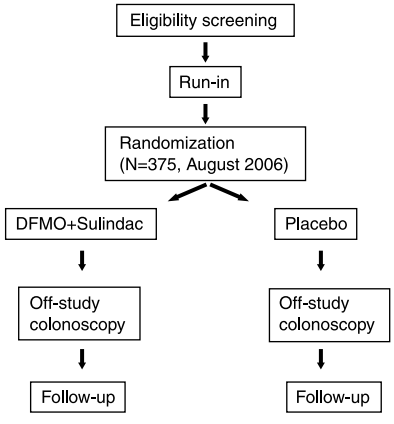

Fig. 2. Study schema for a phase III randomized, double-blind, placebo-controlled clinical trial of the combination of DFMO and sulindac to decrease the rate of recurrence of adenomatous polyps in the colon. As of August 2006, 375 individuals had been randomized to either treatment or placebo arms of this study. Seventy percent of patients are projected to complete the trial as of December 2006 and all participants will have completed the study by 2008

A separate sigmoidoscopy was conducted to collect colorectal biopsies for baseline measures of tissue polyamine and prostaglandin E2 measurements. All participants then entered an eight week pre-trial placebo run-in. This method has been successfully used by our group to minimize participant drop out rates once the actual trial commenced and to ensure participation by participants displaying greater than 70% compliance to treatment guidelines, as measured by pill usage. At the end of the run-in period, participants were randomized, by recruitment site, to either treatment or placebo arms. Treatment was 150 mg Sulindac and 500 mg DFMO per day for 36 months. Placebo was indistinguishable pills for the same duration. By August 2006, 375 participants had been randomized. At the end of 36 months, complete colonoscopy, with polypectomies as necessitated by recurrent polyps, and separate sigmoidoscopy to obtain biopsies for biochemical endpoint assessment were required. These latter procedures were conducted not less than one, and not greater than six, months after the end of treatment. As of December 2006, 205/292 (70%) participants will have completed the trial. All participants are expected to complete the trial by the end of 2008.

Status of other approaches to colon polyp prevention

Several prospective, randomized trials of strategies to prevent colon polyps have been conducted. Two agents, calcium carbonate (Baron et al., 1999) and aspirin (Baron et al., 2003) have been shown to reduce the frequency of polyp recurrence in populations similar to those entered in our studies. Use of either of these agents was associated about a 25% reduction in risk of polyp recurrence. Several trials investigating the efficacy of dietary interventions and physical activity have failed to demonstrate a reduction in colon polyp recurrence risk (Alberts et al., 2000; Schatzkin et al., 2000) in similar participant groups. A recent study of the bile acid ursodeoxycholic acid proved that this intervention did not reduce polyp recurrence, as measured by polyp number, but may have retarded progression of colon polyps as measured by recurrent polyp grade (Alberts et al., 2005). Results from two prospective randomized trials of the COX2 selective agent celecoxib have recently been published (Arber et al., 2006; Bertagnolli et al., 2006). While each trial showed a significant 40–50% reduction in recurrent colon polyps associated with celecoxib treatment, each study found a substantial increase in serious cardiovascular events in individuals taking this drug. Risk-benefit considerations

argue against the use of this agent as a colon cancer preventive agent in humans with an average risk of developing colon cancer (Psaty and Potter, 2006).

Several other agents are currently under evaluation in current phase III clinical trials, including selenium, in the form of a selenized yeast extract, and vitamin D combined with calcium (details of these trials can be found on the web at http://www.cancer.gov/search/ResultsClinicalTrials.aspx?protocolsearchid = 2809065. At this time, results for any agent or combination of agents for chemoprevention will be judged in the context of efficacy, with aspirin and calcium showing the greatest efficacy (~25% reduction in recurrent colon polyps) to date, and safety. In 2006, it is becoming clear that chemoprevention of colon cancer should be part of an overall care and surveillance program, and may have its greatest impact in high risk groups. These groups include those people with high risk, such as FAP, HNPCC family members, other family risk factors and/or people who have had prior large colon polyps and patients with a prior colon cancer. Whether combination chemoprevention with DFMO and sulindac will prove beneficial after consideration along these lines of efficacy and safety remains to be determined.

Acknowledgements

The authors gratefully acknowledge the participation of gastroenterologists who have actively participated in, and contributed to, this study over a number of years. They include: Drs. John McCracken (Long Beach VA Medical Center), Jayashri Kidao (Loma Linda VA Medical Center), Michael Lawson (Kaiser, Sacramento), Dennis Ahnen (University of Colorado University Medical Center and VA), and Kim Turgeon (University of Michigan). We also thank Dr. Christine McLaren for her substantial contributions to the design and analyses of this study to date. This work has been supported by grants and contracts from the US Public Health Service, NIH/NCI CA59024, CA72008, CN-75019, CA88078 and CA95060.

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