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Ornithine Decarboxylase as a Target for Chemoprevention

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Abstract *l*-Ornithine decarboxylase (ODC) is essential for polyamine synthesis and growth in mammalian cells; it provides putrescine that is usually converted into the higher polyamines, spermidine and spermine. Many highly specific and potent inhibitors of ODC are based on the lead compound α-difluoromethylornithine (DFMO), which is an enzyme-activated irreversible inhibitor. DFMO is accepted as a substrate by ODC and is decarboxylated, leading to the formation of a highly reactive species that forms a covalent adduct with either cysteine-360 (90%) or lysine-69 (10%). Both modifications inactivate the enzyme. ODC activity is normally very highly regulated at both transcriptional and post-transcriptional levels according to the growth state of the cell and the intracellular polyamine content. Experimental over-production of ODC can be caused by either transfection with plasmids containing the ODC cDNA with part of the 5'-untranslated region (5'UTR) deleted under the control of a very strong viral promoter, or transfection of plasmids that cause the overproduction of eIF-4E, reported to be a limiting factor in the translation of mRNAs with extensive secondary structures in the 5'UTR. In both cases, unregulated overexpression of ODC transforms NIH 3T3 cells to a neoplastic state. Along with studies showing that many tumor promoters increase ODC activity and that a number of preneoplastic conditions and tumor samples show high levels of ODC, these results suggest that ODC may act as an oncogene in an appropriate background. This provides a rationale for the possible use of ODC inhibitors as chemopreventive agents. Further support comes from studies showing that reducing ODC activity with DFMO abolishes the transformed phenotype of the NIH 3T3 cells overexpressing ODC; many studies found that treatment with DFMO reduces tumor incidence in experimental animals exposed to carcinogens. Although these results provide strong support for initial testing of DFMO as a chemopreventive agent, other means of reducing ODC activity should not be overlooked, including the use of other enzyme-activated irreversible inhibitors with higher potency and/or better pharmacokinetics than DFMO, use of dominant negative mutations or ribzymes to reduce active ODC levels, and use of regulators of ODC expression.

Key words: α-Difluoromethylornithine, enzyme-activated irreversible inhibitors, oncogenes, polyamines, putrescine, tumor promoters

In view of the widespread exposure to cancer-causing environmental stimuli, the hereditary tendency towards neoplastic development in many individuals, and public resistance to simple lifestyle changes that lessen the risk of cancer such as the cessation of smoking, it is of considerable importance to develop safe and effective agents to prevent neoplastic disease. The enzyme *l*-ornithine decarboxylase (ODC) may be an appropriate target for chemoprevention. This article

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summarizes the known biochemistry of ODC, the rationale for its use as a chemopreventive target, available methods to reduce ODC activity, and preliminary studies that evaluate this strategy. Because of space limitations, many important published studies have been omitted in favor of the most recent references and review articles. A more detailed bibliography can be obtained from such articles.

FUNCTION AND REGULATION OF ODC

ODC is the first step in the polyamine biosynthesis pathway. In mammalian cells, it provides the only route for synthesis of putrescine, which is then converted into the higher polyamines spermidine and spermine [1–3]. Reduction of ODC activity would be expected to reduce polyamine levels; however, polyamines are widespread in the diet and are products of intestinal microflora. Mammalian cell membranes contain an active uptake system for polyamines which is stimulated when intracellular polyamines are depleted; uptake of exogenous polyamines ameliorates the effects of ODC reduction.

Very large changes in the ODC activity observed in cells are caused by alterations in the amount of ODC protein. ODC turns over very rapidly, reaching a new steady state of enzyme protein quickly after alterations in the rate of ODC synthesis or breakdown—clearly a growth-associated gene. Many mitogenic stimuli are linked to an increase in ODC activity. Furthermore, a high level of ODC is found in a number of precancerous conditions, many chemical carcinogens have been shown to increase ODC levels, and increased ODC activity is a common feature of exposure to tumor promoters [reviewed in 1–6]. Although the pioneering work showing that ODC responded to tumor promoters was carried out by O'Brien, Boutwell, Verma and their collaborators with 12-*O*-tetradecanoylphorbol-13-acetate (TPA) [4,6 and references therein], many different tumor promoters have now been shown to increase ODC activity. Indeed, elevation of ODC levels has been used as part of a screen for tumor-promoting compounds [5–7]. Transgenic mice expressing the human ODC gene in addition to their endogenous ODC have a much higher level of putrescine in some tissues and show enhanced production of papillomas in response to dimethylbenzanthracene followed by phorbol esters [8]. Several known oncogenes such as c-*myc*, Ki-*ras* and Ha-*ras* (via TGF-β) may increase ODC by enhancing the transcription of the ODC gene [9–13].

Several experiments suggest that ODC may actually act as an oncogene when expressed at very high levels. In two separate studies, a partial cDNA coding for ODC was placed under the control of a strong viral promoter and transfected into NIH 3T3 cells. Resulting clones, which expressed very high levels of ODC, had a malignant phenotype that produced tumors when inoculated into nude mice; the clones also showed anchorage-independent growth in soft agar [14,15]. Although ODC overexpression alone did not produce neoplastic transformation in another study, its combination with the expression of c-Ha-*ras* did produce such transformation [16].

Achieving such high levels of ODC overexpression is difficult because the content of this protein is normally highly regulated by a variety of mechanisms, including changes in the rate of ODC gene transcription, changes in the translation of ODC mRNA, and changes in the rate of degradation [1,2]. In particular, ODC levels are tightly regulated by the cellular polyamine content. Elevated cellular polyamines lead to a reduction in ODC both by inhibiting the translation of ODC mRNA and by increasing production of a protein called antizyme that stimulates degradation of ODC by the 26S proteasome [17].

In many cells, the ODC mRNA is translated inefficiently [1,2,18]. This poor translation appears to be a property of the 5'-untranslated region (5'UTR) of the ODC mRNA. All mammalian ODCs have a long, highly conserved 5'-UTR that contains two features inhibitory to translation: a small open reading frame, which could code for a peptide of about 10 amino acids and probably leads to the dissociation of the ribosome from the mRNA prior to reaching the initiation codon for ODC, and extensive secondary structure [18]. The importance of this secondary structure is emphasized by studies comparing ODC content in NIH 3T3 cells with that in MV7-4E(P2) cells [18–20]. MV7-4E(P2) cells were stably transfected with a plasmid expressing cDNA for the protein synthesis initiation factor eIF-4E [19]. The cells contain high levels of this factor, known to facilitate translation of mRNAs with extensive secondary structure in their 5'-UTRs. The ODC con-

tent in MV7-4E(P2) cells was much greater than in NIH 3T3 cells, particularly later in culture [20]. There was no difference in the content of ODC mRNA between the two cell lines, suggesting that ODC synthesis is increased by the presence of high levels of eIF-4E. Support for this interpretation was obtained by transient transfection of the cells with expression plasmids containing the 5'UTR of ODC attached to the coding sequence of luciferase. These constructs expressed more luciferase in the MV7-4E(P2) cells, whereas control plasmids with the 5'UTR from AdoMetDC or only vector sequences showed no difference in luciferase [18]. Very importantly, the MV7-4E(P2) cells exhibited a transformed phenotype [19], suggesting that ODC overexpression brought about by increased translation may also lead to neoplastic growth [20]. It is certainly possible that levels of other potential oncogene products are also increased in cells overexpressing eIF-4E, but the restoration of a normal phenotype when ODC was reduced by DFMO suggests strongly that ODC is a critical component [20].

The mechanism by which high levels of ODC bring about malignant transformation is likely to involve an increase in intracellular putrescine levels. ODC protein has no known functions except putrescine production, so the simplest interpretation is that the abnormal formation of polyamines leads to malignant growth. This is supported by the finding that DFMO reverses the transformed phenotype of NIH 3T3 cells caused by overexpression of ODC [21] or eIF-4E [20]. No studies have been reported in which the addition of exogenous putrescine transforms cells, but since the uptake system is strongly repressed by intracellular polyamines, it is possible that unregulated expression of ODC is a more effective way of increasing intracellular levels. Addition of putrescine reversed the inhibition of nickel-initiated tumor formation by DFMO [22]. Elevated levels of putrescine may lead to an increased transcription of protooncogenes. Transcription of both c-*fos* and c-*myc* has been increased by putrescine and blocked by DFMO [13 and references therein].

STRUCTURE AND FUNCTION OF ODC

ODC is a pyridoxyl-5'-phosphate (PLP)-dependent enzyme with a subunit MW of about 51,000 Da [1,2,18]. The active enzyme is a homodimer containing two active sites formed at the interface between the dimers [18,23]. Essential residues in mouse ODC (which is extremely similar to the human enzyme and is identical in the key residues described below) include Lys^{69}, the site of PLP binding, Lys^{169}, His^{197} and Cys^{360}. Mutation of any of these amino acids by changing them to alanine causes loss of ODC activity [2, 24]. The active sites are made up of regions that contain Lys^{69}, Lys^{169} and His^{197} from one subunit and Cys^{360} from the other. A most unusual property of ODC is the very rapid and free interchange of subunits under physiological conditions [23]. Therefore, mixing two inactive mutants of ODC, Lys^{69}Ala and Cys^{360}Ala, restores activity by restoring one of the two active sites in the heterodimer to the wild type [18,23]. Furthermore, mixing wild type ODC with a double mutant ODC protein (Lys^{69}Ala/Cys^{360}Ala) causes loss of activity—every heterodimer contains inactivating mutations in both active sites [18,23]. The rapid association and dissociation of ODC subunits may facilitate antizyme binding, which then enhances the degradation of ODC, probably via exposure of the carboxyl terminal domain [18,23]. Deletion of this domain results in an ODC protein that is stable in the cell [reviewed in 2,18].

INHIBITION OF ODC

Many compounds that inhibit ODC have been described [reviewed in 1–3,25,26]. Of particular interest for use as pharmacological agents are the enzyme-activated irreversible inhibitors; DFMO is by far the best known and most widely studied. These compounds were synthesized to act as substrate or product analogues at the active site of the enzyme; their subsequent reaction with the enzyme would generate a reactive species, which would then form a stable covalent addition to the protein, leading to its inactivation. This mechanism has now been fully validated for DFMO [24,27]. Studies using ^{14}C-labelled DFMO have shown that the compound is decarboxylated by ODC and that a stoichiometric amount of DFMO is bound to the enzyme in parallel to the inactivation. Decarboxylation of DFMO followed by elimination of a fluoride anion generates a conjugated imine. This reactive electrophilic imine alkylates the nucleophilic thiol

group of Cys^{360}. Subsequent elimination of another fluoride anion yields a second conjugated imine, which then undergoes a transaldimination reaction with the amino group of Lys^{69}. The corresponding enamine formed by this reaction then cyclizes internally with a concomitant loss of ammonia to give a cyclic imine, *S*-[(2-(1-pyrroline))methyl]cysteine at Cys^{360}. About 90% of the inactivation occurs via this pathway; the major inactivating adduct is therefore at Cys^{360} [27]. The remaining 10% is due to the attachment of DFMO to Lys^{69} [24,27]. The structure of the Lys^{69} adduct has not yet been determined, but it could be formed in a reaction similar to that described above in which lysine is alkylated by the imine, or via a pathway in which the activated form of the inhibitor engages in a nucleophilic attack on the aldimine carbon of the cofactor to initiate the normal transaldimination process leading to product release and reformation of the lysine-PLP Schiff base. The liberated reactive enamine intermediate could then react with the reformed internal aldimine [24].

Although a highly specific inactivator of ODC, DFMO is not particularly potent. The K_i for inactivation is about 40 μM; inactivation proceeds with a half-life of 3.1 min at saturating levels of the drug [2,25,26]. This is a significant fraction of the normal half-life of ODC which is about 20 min, and there is always a fraction of the ODC pool that is not irreversibly inactivated. Furthermore, DFMO is not taken up particularly well by the cell and a large amount of the drug is excreted. Many related compounds that inactivate ODC in a similar way have been synthesized [25,26]. Some of these have significantly better properties for use as ODC inhibitors. Two examples are the methyl ester of (E)-α-monofluoromethyldehydroornithine (Δ-MFMO) and (2R,5R)-δ-methylacetylenicputrescine (MAP). Δ-MFMO has a K_i of only 2.7 μM lower than DFMO; its methyl ester facilitates uptake into the cell where it is converted to the active inhibitor by nonspecific and widely distributed esterases. MAP, in which the α-fluoromethyl group is replaced by an α-acetylenic moiety, is a very effective inhibitor with both a lower K_i of 3 μM and a more rapid rate of inactivation ($t_{1/2}$ of 1.7 min) than DFMO [2,3,25,26]. Other compounds such as the aminoxy derivatives of putrescine, including 3-aminooxy-1-propanamine or 3-(aminooxy)-2-fluoro-1-propanamine, are potent and apparently specific inactivators of ODC which appear to function by binding tightly to the active site [3]. In cell culture experiments they have effects very similar to DFMO but at lower concentrations.

EFFECTS OF ODC INHIBITION ON NEOPLASTIC GROWTH

Many studies found that treatment with DFMO, often well after the initiating agent, greatly reduces tumor development in rodents treated with a wide variety of carcinogens. The production of tumors of the bladder, brain, esophagus, gastrointestinal tract, lung, oral cavity, mammary glands, stomach, skin and trachea [reviewed 1–3,28–31] as well as soft tissues [22], has been blocked by DFMO treatment in animal models. Combining DFMO with other active agents such as oltipraz [31] or piroxicam [32] may lower the doses needed for effective inhibition of tumor development. It is also noteworthy that some other agents or dietary regimes that reduce tumor incidence in animal models may act to reduce ODC levels in target tissues [33,34].

USE OF DFMO AS A CHEMOPREVENTIVE AGENT

The experimental studies described above provide a reasonable case for the use of ODC as a target for chemoprevention. Even though ODC itself is ubiquitous, reduction of excessive ODC levels may have a useful therapeutic effect. This may be brought about by application of specific ODC inhibitors, by dietary manipulations, or by using other compounds that indirectly reduce ODC induction.

By far the most attention has been given to the use of DFMO for this purpose. Several trials have been carried out to determine an appropriate dose of the drug for these studies [35–40]. A significant body of data concerns the potential side effects of DFMO treatment from studies in which this compound has been used as an antitrypanosomal or antitumor agent [2,3]. DFMO is surprisingly non-toxic in view of the potential importance of polyamines for a wide variety of cellular functions, perhaps because of the availability of exogenous polyamines and the difficulties in maintaining a complete blockage of ODC activity due to compensatory increases in activity

and poor uptake of the drug. Thrombocytopenia, anemia, leucopenia and gastrointestinal effects occur in a significant number of patients receiving large doses of DFMO over a prolonged period. A significant number of patients treated with DFMO had loss of hearing. Animal studies have now shown a high level of ODC in the cochlear nerves and that DFMO reduces polyamines in the cochlea. It appears likely that polyamines play an important role in cochlear neurotransmission [2,3]. Such an effect is consistent with extensive literature on modulation of the *N*-methyl-D-aspartate (NMDA) receptor by polyamines [2,3,41]. Most toxic effects of DFMO manifest at the large doses of DFMO needed for antitrypanosomal or anti-tumor effects, which require substantial depletion of polyamines; these effects are readily reversed when the drug is discontinued. The maximal tolerated dose of DFMO in these studies was around 9 g/m^2, but exposures of only 3 g/m^2 led to audiotoxicity in one trial [2,3].

A less complete reduction of ODC may be adequate for chemoprevention since only the enhanced levels of ODC causing development of a malignant phenotype need to be reduced. On the other hand, these trials involve prolonged exposure to the drug. In order to determine an effective dose of DFMO, several endpoints have been used: reduction of TPA-induced ODC activity in skin biopsies [35], reduction in urinary polyamines [36], and the ability to decrease putrescine and the spermidine-to-spermine ratio [37, 38]. Other studies have merely attempted to define the lowest non-toxic dose for prolonged treatments of six months [39] or one year [40]. These studies suggest that daily doses of 0.5 g/m^2 or lower (about 13 mg/kg) should be used to ensure a lack of toxicity, and that these doses do have some effect in diminishing ODC or polyamines. Whether the reduction is sufficient for a useful chemopreventive action remains to be determined.

POSSIBLE PROBLEMS AND FUTURE DIRECTIONS

There is clearly sufficient evidence to support the development of DFMO as a cancer chemopreventive agent. However, a great deal of further experimental work is needed in addition to cautious preliminary trials in appropriate human populations with a high risk of malignant disease development. There is no clear way except for such trials to examine the validity of this approach. Two serious problems are that it is not at all easy to predict the dose of DFMO that will affect tumor incidence, and that it is by no means clear that the very long-term treatment with DFMO will be without toxic effects. Although DFMO itself is relatively non-toxic, its combination with other agents can produce significant synergistic toxicity [2,3]. The antiproliferative action of DFMO is likely to retard the healing of lesions caused by other agents. Such interactions may be difficult to avoid over a prolonged period of treatment. Furthermore, the importance of polyamines for many aspects of cellular physiology is only just beginning to be appreciated. For example, polyamines have a profound effect on the activity of NMDA receptors [41]. Long-term alteration in the activity of these receptors could lead to serious complications.

Although by far the most research has been carried out with DFMO, there are many other ODC inhibitors and little data to support the contention that DFMO is the best one for chemoprevention. Some other available ODC inhibitors may be more suitable properties for the long-term partial reduction of ODC. Use of other non-specific compounds or diets that reduce ODC indirectly are also worthwhile, although evaluating the extent of ODC reduction in such trials is difficult.

Further laboratory examination of the role of ODC in tumor development and promotion will define the extent to which modification of ODC expression leads to neoplastic growth. The use of plasmid constructs containing cDNAs for production of ODC unregulated by the cellular polyamine status will be of great value in such studies. Such constructs may be made by altering the cDNA sequence to delete the 5'-UTR and truncate the amino acid coding sequence at the carboxyl end, providing a more stable ODC. Another possible way to prevent feedback inhibition by polyamines that prevents intracellular accumulation of very large amounts of polyamines would involve the use of plasmid constructs that contain antisense sequences or a ribozyme targeted to reduce the content of antizyme. Similarly, antizyme or ribozyme sequences targeted to ODC or expression of dominant negative double ODC mutants such as $Lys^{69}Ala/Cys^{360}Ala$

can be employed to provide graded reductions in ODC content that could be correlated with the transformed phenotype.

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REFERENCES

1. Pegg AE: Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. Cancer Res 48:759–774, 1988.
2. McCann PP, Pegg AE: Ornithine decarboxylase as an enzyme target for therapy. Pharmacol Ther 54:195–215, 1992.
3. Marton LJ, Pegg AE: Polyamines as targets for therapeutic intervention. Annu Rev Pharm, 1994 (in press).
4. Gilmour SK, Robertson FM, Megosh L, O'Connell SM, Mitchell J, O'Brien TG: Induction of ornithine decarboxylase in specific subpopulations of murine epidermal cells following multiple exposures to 12-*O*-tetradecanoylphorbol-13-acetate, merzerein and ethyl phenylpropriolate. Carcinogenesis 13:51–56, 1992.
5. DiGiovanni J: Multistage carcinogenesis in mouse skin. Pharmacol Ther 54:63–128, 1992.
6. Kim YJ, Pan H, Verma AK: Non-AP-1 tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate-responsive sequences in the human ornithine decarboxylase gene. Mol Carcinogen 10:169–179, 1994.
7. Kitchin KT, Brown JL, Kulkarni AP: Complementarity of genotoxic and nongenotoxic predictors of rodent carcinogenicity. Teratogenesis Carcinog Mutagen 13:83–100, 1994.
8. Halmekytö M, Syrjänen K, Jänne J, Alhonen L: Enhanced papilloma formation in response to skin tumor promotion in transgenic mice overexpressing the human ornithine decarboxylase gene. Biochem Biophys Res Comm 187:493–497, 1992.
9. Bello-Fernandez C, Packham G, Cleveland JL: The ornithine decarboxylase gene is a transcriptional target of c-*myc*. Proc Natl Acad Sci USA 90:7804–7808, 1993.
10. Wagner AJ, Meyers C, Laimins LA, Hay N: c-*myc* induces the expression and activity of ornithine decarboxylase. Cell Growth Differ 4:879–883, 1993.
11. Hurta RAR, Greenberg AH, Wright JA: Transforming growth factor β_1 selectively regulates ornithine decarboxylase gene expression in malignant H-*ras* transformed fibrosarcoma cell lines. J Cell Physiol 156: 272–279, 1993.
12. Wrighton C, Busslinger M: Direct transcriptional stimulation of the ornithine decarboxylase gene by *fos* in PC12 cells but not in fibroblasts. Mol Cell Biol 13:4657–4669, 1993.
13. Tabib A, Bachrach U: Activation of the proto-oncogene c-*myc* and c-*fos* by c-*ras*: Involvement of polyamines. Biochem Biophys Res Comm 202:720–727, 1994.
14. Auvinen M, Paasinen A, Andersson LA, Hölttä E: Ornithine decarboxylase activity is critical for cell transformation. Nature 360:355–358, 1992.
15. Moshier JS, Dosescu J, Skunca M, Luk GD: Transformation of NIH/ 3T3 cells by ornithine decarboxylase overexpression. Cancer Res 53:2618–2622, 1993.
16. Hibshoosh H, Johnson M, Weinstein IB. Effects of overexpression of ornithine decarboxylase (ODC) on growth control and oncogene-induced cell transformation. Oncogene 6:739–743, 1991.
17. Tokunaga F, Goto T, Koide T, Murakami Y, Hayashi S, Tamura T, Tanaka K, Ichihara A. ATP- and antizyme-dependent endoproteolysis of ornithine decarboxylase to oligopeptides by the 26S proteasome. J Biol Chem 269:17382–17385, 1994.
18. Pegg AE, Shantz LM, Coleman CS. Ornithine decarboxylase: Structure, function and translational regulation. Biochem Soc Transact 22, 1994, (in press).
19. Lazaris-Karatzas A, Montine KS, Sonenberg N: Malignant transformation by a eukaryotic initiation factor subunit that binds to mRNA 5' cap. Nature 345:544–547, 1990.
20. Shantz LM, Pegg AE: Overproduction of ornithine decarboxylase caused by relief of translational repression is associated with neoplastic transformation. Cancer Res 54:2313–2316, 1994.
21. Hölltä E, Auvinen M, Andersson LC: Polyamines are essential for cell transformation for $pp60^{v\text{-}src}$: Delineation of molecular events relevant for the transformed phenotype. J Cell Biol 122:903–914, 1993.
22. Ohmori T, Okada K, Tabei R, Shibata T: Effects on tumor induction, growth, metastasis and histology of concurrent administration of putrescine and its metabolizing inhibitor α-difluoromethylornithine in nickel tumorigenesis in soft tissue. Carcinogenesis 15:647–652, 1994.
23. Coleman CS, Stanley BA, Viswanath R, Pegg AE: Rapid exchange of subunits of mammalian ornithine decarboxylase. J Biol Chem 269:3155–3158, 1994.
24. Coleman CS, Stanley BA, Pegg AE: Effect of mutations at active site residues on the activity of ornithine decarboxylase and its inhibition by active site-directed irreversible inhibitors. J Biol Chem 268: 24572–24579, 1993.
25. Sjoerdsma A, Schechter PJ: Chemotherapeutic implications of polyamine biosynthesis inhibition. Clin Pharmacol Ther 35:287–300, 1984.
26. Bey P, Danzin C, Jung M: Inhibition of basic amino acid decarboxylases involved in polyamine biosynthesis. In McCann PP, Pegg AE, Sjoerdsma A (eds): "Inhibition of Polyamine Metabolism. Biological Significance and Basis for New Therapies." Orlando, FL: Academic Press, 1987, pp 1–32.
27. Poulin R, Lu L, Ackermann B, Bey P, Pegg AE: Mechanism of the irreversible inactivation of mouse

ornithine decarboxylase by α-difluoromethylornithine. J Biol Chem 267:150–158, 1992.
28. Kelloff GJ, Malone WF, Boone CW, Sigman CC, Fay JR: Progress in applied chemoprevention research. Semin Oncol 17:438–455, 1990.
29. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC: Chemopreventive drug development: Perspectives and progress. Cancer Epidemiol Biomarkers Prev 3:85–89, 1994.
30. Kelloff GJ, Boone CW, Steele VE, Crowell JA, Lubet R, Sigman CC: Progress in cancer chemoprevention: Perspectives on agent selection and short-term clinical intervention trials. Cancer Res 54:2015s–2024s, 1994.
31. Moon RC, Kelloff GJ, Detrisac CJ, Steele VE, Thomas CF, Sigman CC: Chemoprevention of OH-BBN-induced bladder cancer in mice by oltipraz, alone and in combination with 4-HPR and DFMO. Anticancer Res 14:5–12, 1994.
32. Kulkarni N, Zang E, Kelloff G, Reddy BS: Effect of the chemopreventive agents piroxicam and D,L-α-difluoromethylornithine on intermediate biomarkers of colon carcinogenesis. Carcinogenesis 13:995–1000, 1992.
33. Rao CV, Reddy BS: Modulating effect of amount and types of dietary fat on ornithine decarboxylase, tyrosine protein kinase and prostaglandins production during colon carcinogenesis in male F344 rats. Carcinogenesis 14:1327–1333, 1993.
34. Phillips RW, Kikendall JW, Luk GD, Willis SM, Murphy JR, Maydonovitch C, Bowen PE, Stacewicz-Sapuntzakis M, Wong RKH: β-Carotene inhibits rectal mucosal ornithine decarboxylase activity in colon cancer patients. Cancer Res 53:3723–3725, 1993.
35. Love RR, Carbone PP, Verma AJ, Gilmore D, Carey P, Tusch KD, Pomplun M, Wilding G: Randomized Phase I chemoprevention dose-seeking study of α-difluoromethylornithine. J Natl Cancer Inst 85:732–737, 1993.
36. Pendyala L, Creaven PJ, Porter C: Urinary and erythrocyte polyamines during the evaluation of oral α-difluoromethylornithine in a Phase I chemoprevention clinical trial. Cancer Epidemiol Biomarkers Prev 2:235–241, 1993.
37. Gerner EW, Garewal HS, Emerson SS, Sampliner RE: Gastrointestinal tissue polyamine contents of patients with Barrett's esophagus treated with a α-difluoromethylornithine. Cancer Epidemiol Biomarkers Prev 3:325–330, 1994.
38. Meyskens FL, Emerson SS, Pelot D, Meshkinpour H, Shassetz LR, Einspahr J, Alberts DS, Gerner EW: Dose deescalation chemoprevention trial of α-difluoromethylornithine in patients with colon polyps. J Natl Cancer Inst 86:1122–1130, 1994.
39. Creaven PJ, Pendyala L, Petrelli NJ: Evaluation of α-difluoromethylornithine as a potential chemopreventive agent: Tolerance to daily oral administration in humans. Cancer Epidemiol Biomarkers Prev 2: 243–247, 1993.
40. Crowell JA, Goldenthal EI, Kelloff GJ, Malone WF, Boone CW: Chronic toxicity studies of the potential cancer preventive 2-(difluoromethyl)-*dl*-ornithine. Fundam Appl Toxicol 22:341–354, 1994.
41. Williams K: Mechanisms influencing stimulatory effects of spermine at recombinant *N*-methyl-D-aspartate receptors. Mol Pharmacol 46:161–168, 1994.