Revival of 2-(difluoromethyl)ornithine (DFMO), an inhibitor of polyamine biosynthesis, as a cancer chemopreventive agent

F. Raul

INSERM U682, University Louis Pasteur EA 3430, Laboratory of Nutritional Cancer Prevention, IRCAD, 1 place de l’hôpital, 67091 Strasbourg Cedex, France

Abstract

ODC (ornithine decarboxylase), a key enzyme of polyamine biosynthesis, is an inducible enzyme exhibiting high activity in tumour cells, suggesting ODC as a target for antineoplastic therapy. Among the inhibitors of polyamine-related enzymes, the ODC inactivator DFMO [2-(difluoromethyl)ornithine] became the most well-known. The drug is usually cytostatic and its effects on growth are reversed by micromolar concentrations of polyamines in the cellular environment. ODC inactivation is associated with decreased transcription of the growth-related c-myc and c-fos genes. DFMO used as a single drug has only minor effects on tumour growth. The low efficacy of the drug is due to the use of exogenous (gastrointestinal) polyamines by the mammalian organism. Although it was disappointing in most therapeutic attempts, DFMO showed potential in cancer chemoprevention based on its ability to lower polyamine levels in colorectal mucosa at low dosages with no demonstrable toxicity over long periods of use. DFMO in combination with other drugs prevents and inhibits the development of a variety of chemically induced cancers in animals with doses far lower than those administered for therapy. Low doses of several NSAIDs (non-steroidal anti-inflammatory drugs) and DFMO administered in combination have been shown to be more effective in inhibiting chemically induced colon tumours in rats than are high doses of these agents given individually. This combination has gained further interest after findings suggesting that ODC polymorphism is a genetic marker for colon cancer risk and supporting the use of DFMO and aspirin or other NSAIDs in combination as a strategy for colon cancer prevention.

Introduction

The involvement of polyamines in growth-related processes attracted particular interest as soon as the natural polyamines, putrescine, spermidine and spermine, were recognized as ubiquitous constituents of eukaryotic cells [1–3]. The following observations supported the development of polyamine-related approaches for the treatment of cancer: an excessive urinary excretion of polyamines was reported in cancer patients [4]. Cytotoxic compounds, which alter tumour cell kinetics, were shown also to modify the ability of tumour cells to synthesize and accumulate polyamines [5]. ODC (ornithine decarboxylase), a key enzyme of polyamine biosynthesis, forming putrescine from ornithine, appeared to be an inducible enzyme exhibiting high activity in rapidly growing tissues, particularly in tumour cells, suggesting ODC as a target for antineoplastic therapy [6]. These considerations led investigators at the Merrel Dow Research Institute in Strasbourg (France) to synthesize a series of ornithine derivatives as irreversible ODC inhibitors. From this series of compounds, DFMO [2-(difluoromethyl)ornithine] was selected for development as an anticancer drug [7]. The compound is a rather selective inactivator of ODC and does not inhibit any other enzyme of polyamine metabolism.

Major biological effects of DFMO

Exposure of proliferating cells to 1–5 mM DFMO is followed by a time-dependent decrease in ODC activity and in intracellular putrescine concentration. Depletion of spermidine follows after a lag of a few hours [8]. These changes have been observed in numerous cancer cell lines, and also in tumours and tissues [9,10]. These changes are followed by the impairment of RNA and DNA synthesis. Reduced translation rates, and after prolonged depletion of spermidine, the impairment of hypusine formation, are potential reasons for the impairment of protein synthesis [11]. In human colon carcinoma cells, the inhibition of ODC by DFMO is associated with decreased transcription of the growth-related c-myc and c-fos genes [12]. DFMO is usually cytostatic, and concentrations required for cytotoxicity are greatly in excess of those required to suppress ODC activity. In most cell lines, the effects of DFMO on cell physiology, including growth, are reversed (prevented) by micromolar concentrations of polyamines in the cellular environment of polyamine-depleted cells.
DFMO as a chemotherapeutic agent

DFMO used as a single drug had only minor effects on the growth of EMT-6 solid tumour, and on the survival of L1210 leukemic mice [13]. This was confirmed for a number of different animal tumour models. It appeared that the selective blockade of a single enzyme (ODC) induces changes in metabolism and transport, which compensate for the deficit. Another reason for the low efficacy of DFMO was recognized in the fact that tumours use gastrointestinal polyamines of dietary and bacterial origin. Feeding a polyamine-free diet, combined with the partial decontamination of the gastrointestinal tract with neomycin improved considerably the effect of DFMO on the growth of Lewis lung carcinoma [10].

Early clinical trials with DFMO were disappointing, and, at high doses, several side effects occurred, including diarrhoea, abdominal pain and ototoxicity, which hindered its utility as a cancer therapeutic agent. Clinical trials with a total of 500 patients exhibiting a variety of advanced malignancies showed that DFMO had no significant effect on the progression of the disease [14]. Patients with small-cell lung cancer did not respond favourably to oral treatment, in spite of the sensitivity of small lung carcinoma cells to DFMO in vitro [15]. In a very limited number of trials, stabilization of disease progression was reported occasionally. However these results could not disguise the fact that DFMO as a single drug was in general ineffective in the treatment of malignancies.

However, depending on the tumour type, additive or synergistic anti-tumour activities were reported for combined treatment of experimental animals with DFMO and established chemotherapeutic agents such as cyclophosphamide, Adriamycin, vindesine and BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea] [16–18]. Synergism was also observed more recently between DFMO and all-trans-retinoic acid in xenografts of human colon carcinoma cells in athymic mice [19]. The growth of transplanted tumours was more effectively inhibited by a combination DFMO–indomethacin than by either drug alone [20].

Combined treatment with established cytotoxic agents and DFMO have not been systematically studied in humans, even though combinations of DFMO with BCNU appeared to be promising in the treatment of recurrent glioblastomas [21]. It was shown that a DFMO–interferon α combination suppressed the formation of transplantable mouse melanomas [22]. This observation initiated several clinical trials with disappointing results [23,24]. None of the patients showed objective tumour regression, and the stability of the tumour in a number of patients could not be related to the treatment. In spite of the fact that DFMO has no convincing anticancer effects on established tumours, DFMO still deserves consideration as a promising adjuvant drug in cancer therapy [25]. All of these observations suggest that combinations of DFMO with other drugs could improve existing therapies or lead to new protocols.

DFMO as a chemopreventive agent

Cancer chemoprevention is defined as the use of natural or synthetic (phyto)chemical agents to prevent or inhibit the process of carcinogenesis. The recent interest in DFMO as a chemopreventive agent was advanced by its ability to lower polyamine levels in colorectal mucosa at sufficiently low dosages with minimal or no demonstrable toxicity over long periods of use [26]. The use of mucosal polyamines as a biomarker is supported by its association with colorectal cancer risk [27]. In patients with a history of adenomas, DFMO suppressed the polyamine content of rectal mucosa for 1 year at low doses (0.2–0.4 g/m² per day) in a dose–response manner without a rebound increase of polyamine levels after discontinuation of the drug and without detectable hearing loss or other side effects [28].

Given the complexity and heterogeneity of carcinogenic mechanisms, the rationale for co-administering two or more agents with different modes of action showing synergistic effects while producing minimal toxicity seems compelling. In this context, the application of DFMO–drug combinations in chemoprevention are very attractive. As illustrated in Table 1, DFMO in combination with other (phyto)chemicals prevents the formation and/or inhibits the development of a variety of chemically induced cancers in animals, with doses necessary for preventing carcinogenesis far lower than those administered for cancer therapy.

Efficacy of DFMO in combination for the prevention of colorectal cancer

The association of DFMO with NSAIDs appears of peculiar interest for colorectal cancer prevention. Experimental, clinical and epidemiological evidence indicates that aspirin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Carcinogen</th>
<th>Compound in combination with DFMO</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain, spinal cord, peripheral nerves</td>
<td>Ethylnitrosourea</td>
<td>Retinol, α-tocopherol, thiamine, sodium selenite</td>
<td>[29]</td>
</tr>
<tr>
<td>Intestine (colon)</td>
<td>Dimethylhydrazine</td>
<td>Sodium selenite, mitomycin C</td>
<td>[30,31]</td>
</tr>
<tr>
<td></td>
<td>Azoxy methane</td>
<td>Pirorxicam, fish oil, ellagic acid, oltipraz</td>
<td>[32–35]</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>Methyl nitrosourea</td>
<td>Pirorxicam, aspirin</td>
<td>[36,37]</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Hydroxybutyl nitrosamine</td>
<td>20 candidate chemopreventives</td>
<td>[38]</td>
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</tbody>
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and other NSAIDs prevent the development of colorectal cancer (for a review, see [42]). Low doses of several NSAIDs, including aspirin and DFMO, administered in combination have been shown to be more effective in inhibiting chemically induced colon adenocarcinomas in rats than are high doses of these agents given individually [43]. In addition, tumour formation in the Min (multiple intestinal neoplasia) mouse [which has mutations in the tumour-suppressor gene APC (adenomatous polyposis coli)] was prevented in a significant number of mice by the administration of piroxicam and DFMO [44]. Aspirin and other NSAIDs, by inactivating cyclo-oxygenases and by lowering mucosal prostaglandin concentrations, may have a meaningful impact on polyp development by decreasing both size and number of polyps.

Data from our laboratory (F. Raul, F. Gossé, A.B. Osswald, M. Bouchadjar, C. Foltzer-Jourdaine, J. Marescaux and L. Soler, unpublished work) have shown that a DFMO–aspirin combination given in the drinking water to rats (equivalent to a dose of 0.4 g/m2 per day) starting 5 months after the initiation of colorectal carcinogenesis by a chemical carcinogen, prevented intestinal tumour formation during the 3 months of follow-up by computed tomographic colonography in living rats. During the same period all non-treated rats exhibited colorectal tumour formation. Furthermore, a complete inactivation of ODC associated with a 20% reduction of the polyamine contents, and a 50% decrease of prostaglandin E2 were observed in the colonic mucosa of the DFMO–aspirin-treated rats.

Ongoing clinical trials are also testing the potential synergy between DFMO and various NSAIDs for colorectal cancer prevention [26,45]. This combination has gained further interest after findings confirming the hypothesis that ODC polymorphism is a genetic marker for colon cancer risk and these findings support the use of DFMO and aspirin or other NSAIDs in combination as a strategy for colon cancer prevention [46].

References


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